Spray, O Spray
E.G. Packard, 1906

Spray, farmers, spray with care,
Spray the apple, peach and pear,
Spray for scab, and spray for blight,
Spray, O spray, and do it right.

Spray the scale that's hiding there,
Give the insects all a share,
Let your fruit be smooth and bright,
Spray, O spray, and do it right.

Spray your grapes, spray them well,
Make first class what you've to sell,
The very best is none too good,
You can have it if you would.

Spray your roses, for the slug,
Spray the fat potato bug,
Spray your canteloupes, spray them thin,
You must fight if you would win.

Spray for blight, and spray for rot,
Take good care of what you've got,
Spray, farmers, spray with care,
Spray, O spray the buglets there.

Some Bugs
S. Bjørnson, 1995

Some bugs are bad but most are good,
Some bugs don't want to share our food.
We should all be quite relieved,
That bugs don't always spread disease.

I'll tell you what I've known from youth,
Simply one of nature's truths.
Natural enemies are our friends,
Predators, parasites, and pathogens.

Some bugs lay eggs deep inside,
Other bugs - where their young reside.
Time will pass, the young emerge,
To maintain their status as a scourge.

Some bugs predate on bugs we hate,
Some pests do meet a gruesome fate!
Eaten alive - a predator's role,
A natural means of pest control.

Spray for bugs? Surely you jest,
Sprays kill the good with all the rest.
To kill good bugs is an offence,
Protect good bugs - it makes good sense.

University of Alberta

Morphology and Pathology of the Predatory Mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae)

by

Susan Eleanor Bjørnson ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Department of Entomology

Edmonton, Alberta

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Morphology and Pathology of the Predatory Mite, Phytoseiulus persimilis Athias-Henriot (Acari: Phytoseiidae)* submitted by Susan Eleanor Bjørnson in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Abstract

The predatory mite *Phytoseiulus persimilis* Athias-Henriot is used to control spider mites on a wide variety of crops and is currently one of the most widely used biological control agents in horticulture. Recent reports of poor performance, however, have raised questions regarding the health of this predator.

Predators obtained from several commercial sources were examined for pathogens, specifically microsporidia, and the potential impact of these pathogens on predator performance was evaluated. Internal anatomy of *P. persimilis* was investigated to better describe histopathology. Although not used to describe pathology, the external morphology of *P. persimilis* was also described.

Observed disease signs included abdominal discolouration and rectal plug formation. The former was manifested as two white stripes down the lateral sides of the body in the region of the Malpighian tubules, or as a U-shaped discolouration of the distal opisthosoma. These signs were caused by an accumulation of birefringent, dumbbell-shaped crystals but were not correlated to the presence of potential pathogens observed in *P. persimilis*, including rickettsia, virus-like particles, and microsporidia.

Ultrastructure and pathology of the microsporidium *Microsporidium phytoseiuli* n. sp. were described from *P. persimilis* obtained from a commercial insectary in Europe. Development and pathology of *M. phytoseiuli* were compared to two previously undescribed microsporidia found in *P. persimilis* from North America and Israel.

Phytoseiulus persimilis females infected with *M. phytoseiuli* produced significantly fewer eggs and consumed significantly fewer prey during short-term

performance tests than uninfected adult females from the same source. Survivability during short-term performance tests was highly variable. Lifetime oviposition and longevity of microsporidia-infected females were significantly reduced when compared to uninfected females from a second source. Infected females produced a higher proportion of progeny than uninfected females. Horizontal transmission was low (14.3 percent) during laboratory testing whereas vertical transmission was 100 percent. Adult males did not contribute toward infection of their progeny.

The microsporidium *M. phytoseiuli* was successfully eliminated from colonies using the Pasteur method. The process of rearing infected predators at elevated temperatures did not reduce infection and chemical treatments with albendazole, metronidazole, fumagillin and nifedipine also were ineffective.

Dedication

This thesis is dedicated to Cheryl Marie Bjørnson whose love and friendship has greatly altered the course of my life. I have always admired her unique spirit and sense of optimism.

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Abbreviations

A	anchoring disc
AA	arms of anchoring disc
AB	base of anchoring disc
ABS	ambulacrum stalk
AHS	anterior hypostomatic seta
AL	antero-lateral setae
AMB	ambulacrum
AP	apotele
AS	anal shield
AV	anal valve
C	caeca
CC	cells underlying cuticle
CD	coxal depressors
CH	chelicera
CN	corniculus
CR	cribrum
CS	cone sheath
CTX	cortex (synganglion)
CX	coxa
CXI	coxa, leg I
CXII	coxa, leg II
CXIII	coxa, leg III
CXIV	coxa, leg IV
D	digestive cells
DCH	depressors (extensors) of mobile cheliceral finger
DDP	dorso-lateral dilators of pharynx
DE	developing egg
DP	dorsal pharyngeal constrictors
DPH	dorsal dilators of pharynx
DS	dorsal setae
E	exospore
EN	endospore
ER	rough endoplasmic reticulum
ES	evacuated spore
FD	fixed digit
FM	femur
G	Golgi apparatus
GA	genital atrium
GN	genu
GO	genital orifice
GP	gnathotectal process
GS	genital shield
GT	gnathosoma
HN	host cell nucleus
HP	hypostome
HS	holodorsal shield
IM	internal mala
ISA	inner spermatodactyl arm
LB	labrum
LCH	levator (flexors) of mobile cheliceral finger
LEP	epipharyngeal levators
M	mitochondrion
MD	movable digit
MF	muscle fibre
MP	micropapillae
MS	metapodal shield
MT	Malpighian tubule
MV	microvilli
N	monokaryon
NP	neuropile (synganglion)
OE	oesophagus
OSA	outer spermatodactyl arm
OV	ovarian tissue
P	polaroplast
PAR	paranal seta
PAS	postanal seta
PB	processes at chelicera base
PD	pilus dentilis
PF	polar filament
PHS	posterior hypostomatic setae
PL	postero-lateral seta
PM	plasma membrane
PP	pedipalp
PRS	preanal setae
PS	peritrematic shield
PT	peritreme
PTS	pit sensillum
PV	posterior vacuole
PVL	pulvillus
PX	pharynx
R	ribosomes
RT	rectum
SA	spermatodactyl arm
SB	spermatodactyl base
SCD	subcapitular denticles
SCG	subcapitular groove
SD	spermatodactyl

Abbreviations (continued)

SE	suspensors of endosternite
SG	salivary glands
SGS	sternogenital shield
SN	synganglion
SO	slit organ
SR	seminal receptacle
SS	sternal shield
ST	stigma
STL	salivary stylus
STS	sternal setae
SX	spermatodactyl apex
T	tritosternum
TA	trochanteral abductors
TB	tibia
TC	trachea
TR	trochanter
TS	tarsus
UT	uterus
V	ventriculus
VAS	ventro-anal shield
VDP	ventolateral dilators of pharynx
VP	ventrolateral pharyngeal constrictors
VS	ventral setae
I	leg I
II	leg II
III	leg III
IV	leg IV

Chapter 1
Introduction

Agricultural production and crop pests Serious pest problems are estimated to cause world crop losses of 35 percent, 12 percent attributed to insect and mite damage, 12 percent to plant pathogens, 10 percent to weeds and 1 percent to birds and mammals. In 1991, annual world crop losses due to these pests were estimated at US $400 billion (DeBach and Rosen, 1991). Attempts to reduce crop losses due to insects and other arthropods have been made through various mechanical, cultural, chemical, and biological means; however, chemical control remains the most common strategy for pest control.

In addition to providing pest control on agricultural crops, chemical pesticides have also made a significant contribution toward suppression of arthropod disease vectors and a reduction of disease incidence on a global scale. Despite these obvious benefits, several disadvantages are associated with the use of chemical compounds for pest control, including hazards to non-target organisms (humans, honeybees and natural enemies), persistent residues on food crops and environmental persistence. Pest resurgence is another concern, whereby pests are only temporarily controlled by chemical applications and subsequently become pests of greater magnitude. Yet another concern is the development of new pests by killing the natural enemies of species that were formerly of secondary importance. The broad spectrum effects of many pesticides may cause significant ecological disruptions and further hinder the survival of natural enemies. Furthermore, chemical pesticides have become increasingly more expensive to formulate and apply and chemical applications often provide only a temporary solution for a pest problem (DeBach and Rosen, 1991; Hall, 1991; Solomon and Stephenson, 1991). The misuse or overuse of chemical pesticides are responsible for inducing pest outbreaks (DeBach and Rosen, 1991).

Resistance is considered the greatest problem associated with chemical pesticide use. By 1989, more than 500 arthropod species were reported as resistant to one or more chemical pesticides including 71 species of Acari (Georghiou and Lagunes-Tejeda, 1991). Most reports of pesticide resistance

involve insects that are of agricultural or veterinary importance and this reflects the selection pressure applied against these pest species.

Natural and biological control Natural control, defined as the regulation of populations within certain upper and lower limits over periods of time by natural factors, is considered instrumental for regulation of populations and is thought to be responsible for the control of 99 percent of all potential pests (DeBach and Rosen, 1991). Population reduction of agricultural pests has been achieved by exploiting biological control, a component of natural control whereby actions of natural enemies (parasites, predators, pathogens) help maintain another organism's population at a lower density than would otherwise occur in their absence (DeBach, 1964).

In North America, the first successful classical biological control programme was initiated in California during 1888-1889 to control cottony-cushion scale, *Icerya purchasi* Maskell, a serious pest of citrus. Foreign exploration led to the discovery of a natural enemy, the vedalia beetle, *Rodolia cardinalis* Mulsant, which was imported from Australia and released. All scale infestations were eliminated by 1890 and benefits to the citrus industry have been estimated in millions of dollars annually since control was first achieved (DeBach and Rosen, 1991).

Another outstanding biological control programme was the control of prickly pear (*Opuntia stricta* Haworth and other species) by the lepidopteran *Cactoblastis cactorum* Berg in Australia during the period from 1920 to 1925. These cacti were introduced to Australia's rangeland during the 1800s, and by 1925, 24 million hectares were infested, about half of this land deemed useless for agriculture. Because the cost of chemical control was greater than the value of the land for grazing, most infested land was abandoned. After an intensive search, *C. cactorum* was imported from Argentina and control of prickly pear was achieved within five years following release. Eradication of prickly pear infestations resulted in the return of 24 million hectares of rangeland into production (Holloway, 1964; DeBach and Rosen, 1991). Additional examples of outstanding successes of biological control in recent years have been documented

by DeBach and Rosen (1991).

Biological control in enclosed environments By the early 1900s, biological control for field and forest pests had gained considerable attention, and biological control began to find application in commercial greenhouses. According to Speyer (1927), three *Encarsia* species had been collected and identified from greenhouse whitefly, *Trialeurodes vaporariorum* Westwood during the early 1920s. By 1926, *Encarsia formosa* Gahan was starting to gain recognition for its potential as a biological control agent for greenhouse whitefly in commercial greenhouses (Speyer, 1927). By 1935, 1.5 million wasps were mass-produced annually and shipped to seven countries where they were used for biological control of whitefly in commercial greenhouses. Mass-production continued until 1949, when synthetic insecticides (including DDT) had become available for use by commercial growers. By 1956, there were no *E. formosa* remaining in culture (Hussey, 1985b). In 1960, interest in biological control for greenhouse pests returned after widespread reports of insecticide resistance (Hussey, 1985b).

Pest control within commercial greenhouses is often achieved through the implementation of integrated pest management (IPM) programmes. This approach incorporates all available control measures to reduce pest populations to levels below the economic threshold. This may be accomplished with natural enemies (biological control agents) often in combination with occasional spot treatments of chemical pesticides for severe pest outbreaks.

According to Hussey (1985a), the first integrated programmes were designed to cost one-half that of chemical pest control programmes which at the time often involved more than 20 pesticide applications per crop. Despite the high initial cost associated with biological control agents, costs of an established IPM programme are often less than repeated chemical applications. Reduced costs are due in part to reduced applications of chemical pesticides. An increase in both crop yield and product value are perhaps the most valuable rewards for use of biological control agents. Growers have reported yield increases as high as 25 percent for commercial cucumber crops and public interest in "organically grown" produce (produce grown without chemical pesticide applications) has

increased the market value of many crops (Hussey, 1985a).

More than 80 species of natural enemies are currently under mass production in Europe, with European sales estimated at US $60 million during 1991 (van Lenteren *et al.*, 1997). In 1995, biological control was used for pest control in approximately 70 percent of all commercial greenhouses in Canada. Biological control is used primarily on greenhouse vegetable crops (tomato, cucumber, pepper), and when expressed in terms of total greenhouse area, use of biological control agents is highest in British Columbia (95 percent), followed by Quebec (70 percent), Alberta (67 percent), and Ontario (61 percent) (van Lenteren, 1996). New pest problems in the future will continue the need for further exploration to find effective natural enemies that can be effectively mass-reared and provide adequate pest control.

History and use of Phytoseiulus persimilis In 1960, the predatory mite *Phytoseiulus persimilis* Athias-Henriot was inadvertently transported from Chile to Germany in a shipment of orchids. The ability of *P. persimilis* to control phytophagous mites was apparent, and by 1966 predators were distributed among greenhouses throughout Europe for pest mite control on commercial crops. During these early trials, *P. persimilis* was so effective that pest mites were almost eliminated from treated greenhouses. Use of these predators resulted in significant reductions in acaricide applications and an increase in crop yields. In 1969, commercial production techniques were developed by Mr. J.P. Koppert. *Phytoseiulus persimilis* is currently mass-reared in Australia, Canada, Europe, Israel and the United States and routinely shipped to growers throughout the world (Hussey, 1985b).

Phytoseiulus persimilis are often used for control of two-spotted spider mites, *Tetranychus urticae* Koch, on a wide range of greenhouse crops (Tonks and Everson, 1977; Hamlen and Lindquist, 1981) and to manage pest mite populations in interior settings where insecticide toxicity and residues are of greatest concern (Steiner and Elliott, 1987). In field studies, *P. persimilis* has provided adequate control of red mites on strawberries grown in plastic tunnels and has significantly reduced spider mite populations in corn and soybean (Port and Scopes, 1981;

Schroder, 1983; Pickett and Gilstrap, 1986).

Since its initial discovery over three decades ago, growers have come to depend on *P. persimilis* to provide an effective alternative to chemical pesticides for spider mite control. In 1990, several Alberta growers noted a sharp decline in predator performance and survivability following shipment. Their complaints prompted an investigation at the Alberta Research Council, Vegreville, to determine the cause of reduced efficacy of *P. persimilis*. During this study, numerous predatory mites were observed with abdominal discolouration. Several potential pathogens were found within unidentified tissues of *P. persimilis*, including non-occluded virus-like particles, rickettsia and microsporidia (Steiner, 1993).

Pathogens and biological control agents Although natural enemies, including *P. persimilis*, have demonstrated an ability to provide adequate pest control, there are instances where these same natural enemies provide inadequate control. Numerous explanations have been provided when natural enemies fail; however, few studies directly attribute these failures to disease caused by pathogens. Pathogens can have deleterious effects on host performance and longevity and may prevent the establishment of biological control agents in the field.

Arthropods are hosts for numerous pathogenic bacteria, viruses, fungi and protozoa. Microsporidia are the most common protozoan pathogens of arthropods and can infect both pest and beneficial species, including species under investigation for their biocontrol potential and those currently used as biological control agents (Table 1.1). Most reports of microsporidiosis in beneficial arthropods involve hymenopteran parasitoids which are infected with the same microsporidian species that infects their lepidopteran hosts. Microsporidia infect other natural enemies used for biological control, including other parasitoid groups such as tachinids (York, 1961; Cossentine and Lewis, 1988), predators such as coccinnellids (Lipa and Steinhaus, 1959; Lipa, 1968; Andres and Bennett, 1975; Etzel *et al.*, 1981), entomopathogenic nematodes (Veremtchuk and Issi, 1968), and phytophagous insects used for biological control of weeds (Pettey, 1948; Bucher and Harris, 1961; Andres and Bennett, 1975; Etzel *et al.*, 1981) (Table 1.1).

Microsporidia are responsible for significant reductions in adult emergence, fecundity and longevity of arthropods used as biological control agents (Andreadis, 1980; Etzel *et al.*, 1981; Sajap and Lewis, 1988; Zchori-Fein *et al.*, 1992). Microsporidia have also caused significant host mortality (Peschken and Johnson, 1979). In addition to causing direct deleterious effects, microsporidia are often indirectly responsible for interfering with normal insect development and can retard larval and pupal development of parasitoids. For example, microsporidian spores within the blind gut of *Apanteles* and *Glypta* larvae (parasitoids of spruce budworm, *Choristoneura fumiferana* Clem.) are neither ingested nor able to infect parasitoid tissues. However, spore accumulation in the gut interferes with nutrient acquisition necessary for larval development and pupation (Thomson, 1958). In another study, Cossentine and Lewis (1988) reported that *Lydella thompsoni* Hertig was unable to eclose as adults after development within *Nosema*-infected *Ostrinia nubilalis* Hübner larvae and suggested that spore accumulation within the parasitoid gut is detrimental to parasitoid development.

Microsporidia reduce the reproductive potential of some insects, which may prevent establishment of arthropods released as biological control agents. Peschken and Johnson (1979) reported that copulation and oviposition of *Lema cyanella* L. ceased when infected with *Nosema*. Even when microsporidia-infected insects produce eggs, these are not always viable.

Microsporidia can affect host/parasitoid interrelationships by reducing the number of hosts available for parasitisation. Siegel *et al.* (1986) reported that *Nosema pyrausta* Paillot acted in a delayed density-dependent manner, where high infection levels caused severe population reductions of European corn borer (*O. nubilalis*) and reduced the number of hosts available for parasitisation. *Ostrinia nubilalis* larvae heavily infected with *N. pyrausta* often died before *Lydella thompsoni* parasitoids were able to complete larval development (Cossentine and Lewis, 1988). Weiser (1956) reported that predators of the fall webworm, *Hyphantria cunea* Drury, exhibited selection preferences for microsporidia-infected prey over healthy prey (cited in Laigo and Tamashiro, 1967). In cases where the

same microsporidium can infect both prey and predator, such selection preferences may ultimately reduce the survival of biological control agents in the field. In another study, Rinderer and Elliott (1977) found that microsporidiosis, caused by *Nosema apis* Zander, resulted in aberrant hoarding behaviour of the honeybee *Apis mellifera* L. The effects of microsporidiosis on predator or parasitoid behaviour has not been fully investigated; however, aberrant searching behaviour may further confound efforts to effectively control pests.

Once microsporidia-infected biological control agents are released, the situation may not be altered (Kluge and Caldwell, 1992). The presence of such disease agents may hinder attempts to control plant and arthropod pests in the field (Bucher and Harris, 1961). Biological control agents may be effective when tested in controlled laboratory experiments but less effective in field trials due to a variety of causes, including unfavourable environmental conditions, low reproductive potential and poor searching behaviour. Without routine examination of biological control agents for pathogens prior to their release, it is difficult to determine if the cause of poor performance can be attributed to disease. Diseased arthropods with potential to act as effective biological control agents may be discarded during early stages of testing or lose effectiveness in the field soon after release (Etzel *et al.*, 1981). Microsporidia are known to prevent successful establishment of biological control agents in the field (Sajap and Lewis, 1988) and release of infected beneficials may introduce inoculum into field populations where disease prevalence was otherwise low (Geden *et al.*, 1995) or none previously existed. Infected parasitoids may also serve as vectors through mechanical transmission of microsporidia from diseased to healthy hosts during oviposition (Own and Brooks, 1986). From a research perspective, biological control agents must be examined for pathogens if results from studies regarding life history, predator performance and predator/prey interactions are to be meaningful (Steiner and Bjørnson, 1996).

Pathology of Phytoseiulus persimilis

External morphology and internal anatomy External morphology and internal anatomy of *P. persimilis* are described in Chapter 2. A description of

tissues and internal morphology permits detailed comparisons of healthy and diseased mites. Information regarding internal anatomy and tissues, essential for description of pathology, are described from semi-thin serial sections cut from resin-embedded adult females. Histological sections and transmission electron micrographs are used to provide visual information. External morphology is described for all developmental stages by scanning electron microscopy. Although not used to describe pathological effects of microsporidia, external morphology of *P. persimilis* was investigated to provide information not reported in previous studies.

Abdominal discolouration and rectal plug formation Disease signs observed during this investigation included abdominal discolouration, manifested as two white stripes down the lateral sides of the body in the region of the Malpighian tubules, or a U-shaped discolouration of the distal opisthosoma. These signs, often associated with rectal plug formation, are caused by an accumulation of birefringent dumbbell-shaped crystals. Although abdominal discolouration has been reported in the predatory mites *Amblyseius hibisci* Chant (Tanigoshi *et al.*, 1981), *Neoseiulus* (formerly *Amblyseius*) *cucumeris* Oudemans (Steiner, 1993) and *Metaseiulus occidentalis* Nesbitt (Hess and Hoy, 1982) the cause is not known. Abdominal discolouration and rectal plug formation have been ascribed to senescence (Tanigoshi, 1982); however, some researchers have speculated that abdominal discolourations are diagnostic of pathogens (Schütte *et al.*, 1995). Pathogens previously reported in *P. persimilis* and *M. occidentalis* (see Šuťáková, 1988; Steiner, 1993; Hess and Hoy, 1982) have not been definitively associated with abdominal discolouration or rectal plug formation in these mites.

The investigation reported in Chapter 3 was undertaken to determine whether abdominal discolouration in *P. persimilis* can be attributed to senescence or if pathological signs and associated birefringent crystals are correlated with the presence of potential pathogens observed earlier by Steiner (1993). *Phytoseiulus persimilis* used in this study were obtained from 14 sources between 1990 and 1993: five from Europe, three from North America, three from Australia, two from Israel, and one from New Zealand.

Microsporidia The remainder of this thesis focuses on microsporidian pathogens of *P. persimilis*. In Chapter 4, the ultrastructure and pathology of *Microsporidium phytoseiuli* n. sp. is described from *P. persimilis* obtained from a commercial insectary of Europe in 1993. In Chapter 5, the development and pathology of two previously undescribed microsporidia are described from *P. persimilis* obtained from commercial sources of North America and Israel. Morphological and pathological observations of all three microsporidia are compared.

Effects of M. phytoseiuli on the performance of P. persimilis Recent reports of microsporidia in mass-reared predatory mites *Neoseiulus cucumeris*, *Amblyseius barkeri* Hughes, and *P. persimilis* have raised doubt regarding quality of commercially produced natural enemies (Beerling and van der Geest, 1991; Steiner, 1993; Bjørnson *et al.*, 1996; Steiner and Bjørnson, 1996). Reports of pathogens and concerns regarding performance have resulted in recommendations for quality control guidelines for more than 20 greenhouse biological control agents, including *P. persimilis* (see van Lenteren, 1994). Although microsporidian infections may cause poor performance, aberrant development and reduced survivability in biological control agents (Thomson, 1958; Peschken and Johnson, 1979; Andreadis, 1980; Etzel *et al.*, 1981; Sajap and Lewis, 1988; Zchori-Fein *et al.*, 1992), past relationships linking microsporidia to poor performance in mass-reared mites has been based on circumstantial evidence.

The effects of *M. phytoseiuli* on performance of *P. persimilis* are described in Chapter 6. Short-term performance tests were used to evaluate prey consumption, oviposition rates and survivability of uninfected and microsporidia-infected predators. Results were compared to minimal proposed standards outlined in current quality control guidelines (van Lenteren, 1994). Uninfected and microsporidia-infected *P. persimilis* adult females were also evaluated for lifetime oviposition and longevity.

Transmission Pathogen transmission and disease prevalence of *M. phytoseiuli* are reported in Chapter 7. Horizontal and vertical transmission, and parental contribution toward infection were investigated.

Cultural and chemical control Cultural and chemical methods were evaluated for control of microsporidiosis in *P. persimilis* and are reported in Chapter 8. Attempts were made to control microsporidia by pathogen exclusion (the Pasteur method), rearing predators above temperatures that favour optimal development and through the application of antimicrosporidial compounds (albendazole, metronidazole, fumagillin and nifedipine).

Table 1.1
Summary of Potential or Current Biological Control Agents Infected with Microsporidia

Authors	Beneficial arthropod/nematode	Type	Host
Allen and Brunson (1945)	*Macrocentrus ancylivorus* Rohwer (Hym.)	parasitoid	*Gnorimoschema operculella* Zell. (Lep.)
Pettey (1948)	*Cactoblastis cactorum* Berg (Lep.)	phytophagous	*Opuntia* spp. (Cactaceae)
Blunck (1954)	*Apanteles glomeratus* L. (Hym.)	parasitoid	*Pieris* spp.; *Aporia* spp.
	Haplaspis nanus Grav. (Hym.)	parasitoid	
	Hemiteles simillimus sulcatus Bl. (Hym.)	parasitoid	
	Gelis cf. *transfuga* Först (Hym.)	parasitoid	
	Thysiotorus brevis Thoms. (Hym.)	parasitoid	
	Dibrachys cavus Walk. (Hym.)	parasitoid	
	Tetrastichus rapo Walk. (Hym.)	parasitoid	
Tanada (1955)	*Apanteles glomeratus*	parasitoid	*Pieris brassicae* L. (Lep.)
Thomson (1958)	*Apanteles fumiferanae*† Viereck (Hym.)	parasitoid	*Choristoneura fumiferana* Clem. (Lep.)
	Glypta fumiferanae† Viereck (Hym.)	parasitoid	
Lipa and Steinhaus (1959)	*Hippodamia convergens* Guérin (Col.)	predator	homopterous insects
Bucher and Harris (1961)	*Hypocrita jacobaeae* L. (Lep.)	phytophagous	*Senecio jacobaea* L. (Asteraceae)
York (1961)	*Chelonus annulipes* Wesmael (Hym.)	parasitoid	*Pyrausta* (*Ostrinia*) *nubilalis* Hübner (Lep.)
	Lydella grisescens Robineau-Desvoidy (Dip.)	parasitoid	
	Macrocentrus gifuensis Ashmead (Hym.)	parasitoid	
Issi and Maslennikova (1966)	*Apanteles glomeratus*	parasitoid	*Pieris brassicae* (Lep.)
Laigo and Tamashiro (1967)	*Apanteles marginiventris* Cresson (Hym.)	parasitoid	*Spodoptera mauritia acronyctoides* Guenee (Lep.)
Lipa (1968)	*Coccinella semipunctata* L. (Col.)	predator	homopterous insects
	Hippodamia tredecimpunctata L. (Col.)	predator	homopterous insects
	Myrrha octodecimguttata L. (Col.)	predator	homopterous insects
Veremtchuk and Issi (1968)	*Neoaplectana* spp. (Stein.)	parasite	generalist - many insect species
Hostounský (1970)	*Apanteles glomeratus*	parasitoid	*Pieris brassicae*
	Hyposoter ebeninus Grav. (Hym.)	parasitoid	
	Pimpla instigator F. (Hym.)	parasitoid	
Smirnoff (1971)	*Dahlbominus fuscipennis* Zett. (Hym.)	parasitoid	*Neodiprion* spp.; *Pristiphora erichsonii* Htg. (Lep.)

Table 1.1 (continued)
Summary of Potential or Current Biological Control Agents Infected with Microsporidia

Authors	Beneficial arthropod/nematode	Type	Host
Brooks and Cranford (1972)	*Campoletis sonorensis* Cameron (Hym.)	parasitoid	*Heliothis zea* Boddie (Lep.); *H. virescens* F. (Lep.)
	Cardiochiles nigriceps Viereck (Hym.)	parasitoid	
Andres and Bennett (1975)	*Acigona infusella* Walker (Lep.)	phytophagous	*Eichhornia crassipes* (Mart) Solms (Pontederiaceae)
	Epipagis albiguttalis Hmps. (Lep.)	phytophagous	
	Neochetina eichhorniae Warner (Col.)	phytophagous	
	Neochetina bruchi Hustache (Col.)	phytophagous	
Bell and McGovern (1975)	*Bracon mellitor* Say (Hym.)	parasitoid	*Anthonomus grandis* Boheman (Col.)
Peschken and Johnson (1979)	*Lema cyanella* L. (Col.)	phytophagous	*Cirsium arvense* L. (Asteraceae)
Andreadis (1980)	*Macrocentrus grandii* Goidanich (Hym.)	parasitoid	*Ostrinia nubilalis* Hübner
Etzel *et. al.* (1981)	*Galeruca rufa* Germar (Col.)	phytophagous	*Convolvulus arvensis* L. (Convolvulaceae)
	Rhinocyllus conicus Froelich (Col.)	phytophagous	*Carduus natans* L. (Asteraceae)
	Ceutorhynchidius horridus Panzer (Col.)	phytophagous	
	Ceutorhynchus trimaculatus F. (Col.)	phytophagous	
	Coleophora parthenica Meyrick (Lep.)	phytophagous	*Halogeton glomeratus* C.A. Meyer (Chenopodaceae)
Huger (1984)	*Trichogramma evanescens* Westwood (Hym.)	parasitoid	*Ostrinia nubilalis*
Cossentine and Lewis (1988)	*Lydella thompsoni*† Herting (Dip.)	parasitoid	*Ostrinia nubilalis*
Sajap and Lewis (1988)	*Trichogramma nubilale* Ertle & Davis (Hym.)	parasitoid	*Ostrinia nubilalis*
Own and Brooks (1986)	*Pediobius foveolatus* Crawford (Hym.)	parasitoid	*Epilachna varivestis* Mulsant (Col.)
Zchori-Fein *et al.* (1992)	*Muscidifurax raptor* Girault & Sanders (Hym.)	parasitoid	*Musca domestica* L.; *Stomoxys calcitrans* L. (Dip.)
Beerling *et al.* (1993)	*Amblyseius cucumeris* Oudemans (Acari)	predator	*Frankliniella occidentalis* Pergande (Thys.)
	Amblyseius barkeri Hughes (Acari)	predator	*Thrips tabaci* Lindeman (Thys.)
Bjørnson *et al.* (1996)	*Phytoseiulus persimilis* Athias-Henriot (Acari)	predator	*Tetranychus urticae* Koch (Acari)

†, gut infection without evidence of parasite invasion into tissues. Col., Coleoptera; Dip, Diptera; Hym., Hymenoptera; Lep., Lepidoptera; Stein., Steinernematidae; Thys, Thysanoptera.

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Chapter 2
External and Internal Morphology of the Predatory Mite, *Phytoseiulus persimilis* (Acari: Phytoseiidae)

Introduction

Mites of the Family Phytoseiidae occur in diverse ecosystems throughout the world, ranging from arctic tundra to tropical forests. These free-living, terrestrial mites occupy a variety of habitats and are found on foliage, leaf litter, bark or humus where they feed on small arthropods, homopteran honeydew, pollen and, rarely, on plants (Chant, 1985; Gerson and Smiley, 1990). Many species that feed on tetranychid mites or other crop pests have been studied in detail. Some of these are used for pest control within agroecosystems, including members of the genera *Phytoseiulus*, *Amblyseius* and *Metaseiulus* (see Gerson and Smiley, 1990).

Phytoseiulus persimilis is currently used throughout the world for pest mite control on greenhouse, orchard, horticultural and agricultural field crops such as corn, soybean and strawberries. Widespread use of *P. persimilis* has established this predatory phytoseiid as the most available natural enemy produced within European commercial insectaries (van Lenteren *et al.*, 1997).

Previous research on *P. persimilis* has focused primarily on life history, predator/prey interactions and pest control dynamics, while few studies have investigated the external morphology or internal anatomy of this predator. Hessein (1976) described external morphology and provided illustrations of both dorsal and ventral shields and arrangements of setae for all developmental stages. In a separate study, Akimov and Yastrebtsov (1987) described the gnathosomal, pharyngeal and opisthosomal muscles and their attachment sites. Although frontal tissue sections were included in this study, most tissues were not identified. In other studies, only the digestive system of *P. persimilis* has been investigated in any detail (Akimov and Starovir, 1974, 1978). Detailed histological studies of the reproductive system are lacking. Despite previous investigations to assess reproductive potential (Amano and Chant, 1977; Eveleigh and Chant, 1981), the means that enable ovipositional *P. persimilis* females to produce several eggs per day, each approximately half the size of the adult female (Hessein, 1976), remains

poorly understood (Chant, 1985).

With few exceptions, illustrations used in previous studies to portray morphological and anatomical features of *P. persimilis* are based on light microscopic observations. Although these studies provide useful morphological observations, resolution of detail is often inadequate. Important information may be overlooked and observations are difficult to interpret at this level of resolution.

Limited information is currently available to assist with tissue identification either by light or transmission electron microscopy. The purpose of this study was to describe normal *P. persimilis* tissues and internal anatomy so that comparisons with internal anatomy and tissues of diseased mites could be made. Histological descriptions are based on examinations of semi-thin serial sections cut from whole mites embedded in Spurr resin. Morphological descriptions are based on interpretations of semi-thin tissue sections observed by light and transmission electron microscopy. Micrographs accompany these descriptions when possible. External morphology of all developmental stages was examined by scanning electron microscopy.

Materials and Methods

Mite rearing *Phytoseiulus persimilis* for this study were obtained from a supplier of biological control agents in New Zealand (June 1993) and Europe (December 1993). Mite colonies were reared within cages at the Alberta Research Council, Vegreville, where they were maintained on bean plants (*Phaseolus vulgaris* L.) infested with two-spotted spider mites, *Tetranychus urticae*. Cages were placed within greenhouses (18L:6D; 25°C:20°C) and predators were randomly sampled and examined periodically to ensure that colonies were free of microsporidian pathogens.

Light microscopy Light microscopic preparations used to observe external morphology were made by placing individual mites within a drop of distilled water and gently applying a coverslip. For observation of internal tissues, semi-thin serial sections, approximately 1-1.5 μm in thickness, were cut from whole, adult female mites embedded in Spurr resin (see below).

Glass knives and an LKB Nova Ultramicrotome were used to cut semi-thin

sections. Glass knives were equipped with 'boats' to hold distilled water during sectioning. These were constructed by placing a strip of metallic tape adjacent to one side of the blade edge and carefully wrapping the tape around the front of the blade toward the other side of the blade edge. The tape base in contact with the bevelled glass was sealed with two coats of nail polish. A Pasteur pipette formed into a glass rod was used to remove serial sections as they were cut. These were placed on the surface of a drop of distilled water on APTS- (3-aminopropyltriethoxysilane) coated slides, dried on a hot plate and stained with 1% toluidine blue in borax buffer (pH 7.4).

Scanning electron microscopy (SEM) Uninfected predators from the European source (source EU2) were removed from leaf surfaces using a fine bristle paint brush and placed in 70% ethanol for 24 hours. Specimens were dehydrated in the following series of ethanol: 80% (10 min), 85% (10 min), 90% (10 min), 95% (10 min), 100% (20 min), followed by amyl acetate: absolute ethanol (1:1) (10 min), amyl acetate: absolute ethanol (2:1) (10 min) and 100% amyl acetate (24 h). Specimens were processed within Teflon capsules throughout this procedure. Following critical point drying, specimens were mounted on stubs covered with carbon tape, gold-coated and examined using a JOEL JSM.6301FXV field emission scanning electron microscope (accelerating voltage, 5 kV). Body measurements were taken directly from scanning electron micrographs; length was measured from the tip of the gnathosoma to the tip of the distal opisthosoma and width measurements were taken at the widest part of the idiosoma.

Some predators were examined with cryo SEM. Portions of bean leaves with predators were placed lower surface up on cryo stubs. Leaves were affixed to stubs with O.C.T. compound (cryo embedding media for frozen tissue specimens). Stubs were submerged in liquid nitrogen previously cooled to its freezing point (-207°C). Ice was sublimed from specimens which were then gold-coated and examined with a JOEL JSM.6301FXV field emission scanning electron microscope (accelerating voltage, 5 kV).

Transmission electron microscopy (TEM) Adult female *P. persimilis* (from New Zealand) were placed in fixative consisting of 1% paraformaldehyde and

1.5% glutaraldehyde in cacodylate buffer for 24 to 48 hrs. Fixative was replaced with 0.12 M cacodylate buffer (pH 7.2) with three changes in 60 min. Tissue was postfixed for two hours in 1% osmium tetroxide in 0.12 M cacodylate buffer.

Following fixation, mites were placed in distilled water for 10 min and dehydrated in the following ethanol series: 50% (30 min), 70% (30 min), 90% (30 min), 100% (60 min), followed by propylene oxide: absolute ethanol (1:1) (30 min), propylene oxide (60 min), propylene oxide:low viscosity Spurr resin (16 h), and low viscosity Spurr resin (24 h). Each solution was changed three times. An individual mite was placed in Spurr resin within a flat mould and cured for a minimum of 16 hrs in a 60°C oven.

Ultrathin gold sections, approximately 100 nm thick, were cut with a diamond knife using an LKB Nova Ultramicrotome. Sections were placed on Formvar-coated grids and stained with prefiltered, 4% uranyl acetate for 20 min in a 60°C oven, then with lead citrate for 6 min at room temperature. Sections were examined with an Hitachi H-600 transmission electron microscope (accelerating voltage, 75 kV).

Results

Morphological structures were identified and labelled according to information derived from Evans (1992). Muscles (gnathosomal, cheliceral, pharyngeal and opisthosomal) were identified and labelled according to descriptions by Starovir (1973) and Akimov and Yastrebtsov (1987). Internal tissues were identified and described according to diagrammatical information included in previous studies of *P. persimilis* (see Starovir, 1973; Akimov and Starovir, 1974, 1978; Arutunjan, 1985) and other mesostigmatid mites (Winkler, 1888; Michael, 1892; Jakeman, 1961; Woodring and Galbraith, 1976).

External morphology The body is divided into two major regions, the anterior gnathosoma and the posterior idiosoma (Krantz, 1978). The idiosoma is divided into two sections, the anterior region referred to as the podosoma (the portion of the idiosoma to which the legs are attached) and the more distal opisthosoma. Larvae are readily distinguished from other stages by the presence of three pairs of legs; all other stages have four pairs. Legs were numbered in

descending order from either side of the gnathosoma toward the distal opisthosoma and are referred to as legs I, II, III and IV.

External morphology of all five developmental stages of *P. persimilis* is described below in the following order: egg, larva, protonymph, deuteronymph and adult. Adult males and females are described separately. A general description of the legs and pedipalps of immatures and adults is provided, followed by a description of the gnathosoma of all life stages.

Egg Eggs are oval and measure 219.23±2.73 x 178±1.48 μm (n=10) (Table 2.1). The egg is translucent or light beige during the first 24 hr of development and turns light pink or orange to dark orange as it matures. Striations were often observed through the chorion of older eggs prior to larval emergence.

Larva Larvae are oval and measure 233.06±7.86 x 141.02±4.77 μm (n=5) (Table 2.1). A dorsal view of a larva is shown in Figure 2.1. A composite diagram of the dorsal surface of a larva illustrates the arrangement of the 10 pairs of dorsal setae (Figure 2.2). A lightly sclerotized shield was observed on the dorsal surface with SEM. The dorsal shield appears as a slightly elevated ridge that extends from the first anterior pair of dorsal setae toward the posterior opisthosoma and surrounds the ninth pair of dorsal setae. Well-developed chelicerae were observed by light microscopy and consist of fixed and moveable digits, the former with a dentate ventral surface.

A ventral view of a larva is shown in Figure 2.3. Ventral shields (both sternal and anal) are not present. A diagram of the ventral surface outlines the position of ventral setae including: three pairs of sternal setae (STS; positioned on the ventral podosomal region between coxae of legs I and III), three pairs of preanal setae (PRS), one pair of paranal setae (PAR) and a single postanal seta (PAS) (Figure 2.4). A single pair of ventral pores are located posterior to the third pair of sternal setae (Figures 2.3 and 2.5). These were observed only on the ventral surface of larvae and are absent from other life stages. The anal opening is comprised of a pair of anal valves (AV, Figure 2.6). A second pair of ventral pores are located near the anus; each pore is located on the outer side of each paranal seta (Figure 2.6, arrowheads). These pores are conspicuous and were

observed only on immature stages (larvae, protonymphs and deuteronymphs).

Protonymph Protonymphs measure 258.44±7.64 x 143.04±5.98 μm (n=5) (Table 2.1). The dorsal surface of each protonymph has a lightly sclerotized dorsal shield and 16 pairs of dorsal setae. Protonymph chelicerae were observed only by light microscopy and are similar in appearance to larval chelicerae. A short peritreme extends from the coxa of leg I toward the anterior region of the body.

Protonymphs are morphologically similar to *P. persimilis* deuteronymphs. Ventral shields (sternal and anal) are absent; however, a sulcate pattern was observed on the lateral and ventral surfaces of the body. This pattern extends ventrally from the posterior podosoma toward the anus and appears more prominent in protonymphs that shrivelled excessively during specimen preparation. The ventral setae include three pairs of sternal setae (podosoma), three pairs of preanal setae (opisthosoma), a pair of paranal setae and a single postanal seta (distal opisthosoma, adjacent to anus). The anus, paranal setae and postanal seta are located on a smooth surface at the tip of the distal opisthosoma. Two anal pores were observed on the perimeter of the smooth surface surrounding the anus, one on the outer side of each paranal seta. Because *P. persimilis* protonymphs are morphologically similar to deuteronymphs, only micrographs of deuteronymphs were included in this study.

Deuteronymph Deuteronymphs measure 293.83±6.59 x 161.83±7.89 μm (n=3) (Table 2.1). A dorsal view of a deuteronymph is shown in Figure 2.7. A composite diagram of the dorsal surface shows the lightly sclerotized dorsal shield and arrangement of the 16 pairs of dorsal setae (Figure 2.8).

The chelicerae are well developed (Figure 2.9). The ventral surface of the fixed digit (FD) is dentate whereas the moveable digit (MD) is comprised of a single tooth-like structure that curves upward toward the fixed digit. The fixed digit of each chelicera bears two sensilla, including a medial trichoid sensillum, the pilus dentilis (PD), and the apical pit sensillum (PTS) (Figure 2.9 inset). When viewed dorsally, a teardrop-shaped dorsal seta (arrowhead) is visible near the basal region of each fixed digit, on the outer side of the dorsal slit organ (SO,

Figure 2.10). A second slit organ is positioned laterally on the outer side of the cheliceral arm, posterior to the base of the fixed digit (Figure 2.9). Arthrodial membrane at the base of the moveable digit (PB) consists of a group of setiform processes on the ventral surface of each chelicera. The ventral surface of fully extended chelicerae are shown in Figure 2.11. A ventral view of the hypostome (HP) shows the pedipalps (TR, FM) and partially extended chelicerae (CH) (Figure 2.12).

A ventral view of the gnathosoma in Figure 2.13 illustrates the position of the pedipalps (PP), chelicerae and tritosternum (T). The bifurcate tritosternum arises ventrally near the gnathosomatic base and is directed anteriorly, often concealing the subcapitular groove (SCG, Figure 2.13). The tritosternum is present on all developmental stages. Anteriorly directed, subcapitular denticles are arranged in transverse rows within the subcapitular groove.

The ventral podosomal region of the deuteronymph possesses five pairs of sternal setae positioned between coxae of legs I and IV (Figure 2.14). Posterior to the sternal setae are three pairs of preanal setae (Figure 2.15), followed by one pair of paranal setae and a single postanal seta (Figure 2.16). Ventral shields (sternal or anal) are not present. The posterior opisthosoma has a lightly sculptured surface; the sulcate pattern extends from the most posterior pair of sternal setae toward the anus and surrounds the anus, paranal setae and post anal seta. The cribrum (CR) is posterior to the anus (Figure 2.16) and two pores are positioned on the perimeter of the smooth surface that surrounds the anus, one on the outer side of each paranal seta (Figure 2.16, arrowheads).

Adult male Adult males measure 323.60±16.82 x 188.28±2.42 μm (n=4) (Table 2.1). The dorsal surface of an adult male is shown in Figure 2.17. A composite diagram of the dorsal shield and arrangement of 16 pairs of dorsal setae are shown in Figure 2.18.

The chelicerae of the male are shown retracted under the gnathotectal process (GP), the anterior portion of the dorsal cheliceral frame (Figure 2.19), and partially extended over the hypostome (Figure 2.20). Each fixed digit has a dentate ventral surface, an apical pore-like pit sensillum (not shown), and two

trichoid sensilla including a teardrop-shaped dorsal sensillum and a medial trichoid sensillum (pilus dentilis) (Figure 2.21). Each moveable digit consists of a single tooth-like spine that curves sharply upward; the apex of each moveable digit appears to fit within a depression on the outer surface of the corresponding fixed digit (Figure 2.19).

Numbers and arrangement of slit organs on adult male chelicerae are similar to those observed on deuteronymph chelicerae. A teardrop-shaped dorsal seta is located near the basal region of each fixed digit on the lateral side of the dorsal slit organ (Figure 2.21, arrowhead). A second slit organ was observed on the lateral sides of each chelicera, posterior to the spermatodactyl base (not shown).

Each spermatodactyl arm (SA) is broad and extends anteriorly from the spermatodactyl base (SB). A groove along the inside of each spermatodactyl arm gives the appearance that each is partitioned into an inner (ISA) and outer (OSA) region (Figure 2.21 inset). The outer region terminates in an outward pointing horn-like projection; the inner partition of the spermatodactyl arm projects beyond the outer region and extends anteriorly at a downward angle (Figure 2.21). A horn-like projection on the inner portion of the spermatodactyl arm points inward. The inner partition of the spermatodactyl arm ends with a broad, pad-like apex (SX, Figures 2.22 and 2.22 inset). A single pore is located on each apex.

The sternogenital shield (SGS) is shown in Figure 2.23. A diagram of the sternogenital shield (Figure 2.24) shows the position of the five pairs of sternal setae and male genital orifice (GO); the latter is located on the anterior region of the sternogenital shield. The genital orifice is covered by an m-shaped anterior extension of the sternogenital shield (Figure 2.25). Two structures (one with two points and the other resembling a deflated bag) extend from the genital orifice on some males (Figure 2.26, arrowheads) and may be either part of the male reproductive system or a portion of the spermatophore. The position of the ventro-anal shield (VAS) is shown in Figure 2.27 and a diagram of the ventro-anal shield illustrates the position of ventral opisthosomal setae and anus (Figure 2.28).

The surfaces of the sternogenital and ventro-anal shields are sculpted in a reticulate pattern (Figures 2.23 and 2.27). Surface sculpturing was observed by light microscopy and the ventral shields could be readily identified.

Adult female Adult females measure 468.04±4.09 x 293.44±4.56 μm (n=5) (Table 2.1). The dorsal surface of an adult female is shown in Figure 2.29. The holodorsal shield (HS) is highly sclerotized. A composite diagram of holodorsal shield shows the relative size and arrangement of the 16 pairs of dorsal setae (Figure 2.30). The longer dorsal setae of the adult female and all other developmental stages except the larvae are bristled (Figure 2.31). Two dome-like structures are located on the holodorsal shield (Figure 2.31 inset) posterior to the first pair of anterior dorsal setae and between the second pair of dorsal setae. The dorsal shield is sculptured with a reticulate-like pattern; a sulcate pattern surrounds the shield and extends toward and surrounds the ventral shields.

Stigmata (ST) are located on the podosomatic region dorsolateral to coxae of legs III and IV. The peritreme (PT), an anteriorly directed groove, is associated with each stigma and extends from the stigma toward the dorsolateral side of leg II (Figure 2.32). The peritreme contains tuberculate micropapillae (MP, Figure 2.32 inset) and is surrounded by the peritrematic shield (PS).

The chelicerae of the adult female are shown extended over the hypostome (Figure 2.33). Teardrop-shaped setae located on the dorsal surface of the chelicera are similar in appearance to those on the dorsal surface of adult male and deuteronymph chelicerae (see Figures 2.10 and 2.21). Each chelicera is comprised of fixed and moveable digits and although the ventral surface of the fixed digit is dentate, the tooth-like projections are less prominent than those on the fixed digit of adult male chelicerae (see Figure 2.21). A medial trichoid sensillum (pilus dentilis) and apical pit sensillum are located on each fixed digit (Figure 2.34). Two slit organs (dorsal and lateral) are present and similar in appearance to those on the chelicera of deuteronymphs and adult males (not shown). The moveable digit is formed into a single tooth-like spine that extends upward to a lesser extent than that of the male. Arthrodial membrane at the base of the moveable digit appears as a group of setiform processes on the ventral

surface of each chelicera as described previously for the deuteronymph.

A lateral view of an adult female shows the position of the stigma and peritreme in reference to the holodorsal shield and legs (Figure 2.35). The metapodal shield is located on the lateral side of the body, posterior to coxae of leg IV. The metapodal shield (MS) appears on either side of the body as two slight impressions on an otherwise immaculately sculptured surface (Figure 2.36).

The sternal shield extends posteriorly from the base of the gnathosoma to the posterior side of leg II (Figure 2.37). A diagram shows the arrangement of the three pairs of sternal setae along the lateral sides of the sternal shield (SS, Figure 2.38). The genital shield is posterior to the sternal shield and extends from the medial area of leg III toward the distal opisthosoma (Figure 2.39). The genital shield is pear-shaped and sulcate ridges are present on the lateral and distal edges. A diagram of the genital shield shows the relative position of the genital orifice and the position of the two ventral setae located on the lower half of the genital shield (Figure 2.40).

The genital orifice extends beyond either side of the anterior region of the genital shield (GS) and appears to be more prominent in some females (Figure 2.41). A rounded object protruding from the genital orifice of one female may be part of a spermatophore (Figure 2.42, arrowhead). Four pairs of preanal setae were located on the sulcate surface of the distal opisthosoma between the genital and anal shields (Figure 2.43). The anus (comprised of two anal valves), a pair of paranal setae and a single postanal seta are located on the anal shield at the posterior tip of the distal opisthosoma (Figure 2.44). For all life stages, none of the ventral setae are bristled. The anal shield has a smoother surface than the surrounding distal opisthosoma and was readily observed by light microscopy whereas the sternal and genital shields were more difficult to discern.

Legs Larvae have three pairs of legs; all other stages have four pairs. The legs are comprised of seven podomeres (from proximal to distal): coxa (CX), trochanter (TR), femur (FM), genu (GN), tibia (TB), tarsus (TS), and apotele (Figure 2.45). The apotele, represented by the claw complex is located on the apex of the ambulacral stalk (ABS, Figure 2.46). A dentate process extends from

the base of the ambulacral stalk which is otherwise covered with deeply ridged, longitudinally arranged scale-like processes. The claws and the scale-like processes of the ambulacrum are used to grasp web silk produced by the prey and allows *P. persimilis* to walk with agility on prey webbing (Figures 2.47 and 2.47 inset).

The gnathosoma

The gnathosoma is comprised of a pair of pedipalps, a pair of chelicerae and the basal subcapitulum which forms the ventral surface of the gnathosoma. The chelicerae have been described earlier with each developmental stage and are not redescribed here.

Pedipalps A pair of pedipalps are located on the external side of the chelicerae. Each pedipalp is composed of six podomeres (from proximal to distal): the trochanter, femur, genu, tibia, tarsus, and apotele (AP, Figure 2.48). The apotele is located on the interior edge of each tarsus and appears as a somewhat flattened, claw-like structure (Figure 2.49). Numerous setae are positioned on the dorsal, ventral and lateral surfaces of the pedipalps and most are setiform, with exception of the peg-like antero-lateral setae (AL). Several setiform sensilla are located on the apex of each tarsus (Figure 2.49).

Larval gnathosoma The larval gnathosoma is distinct from the gnathosoma of other life stages. The subcapitular groove of the larval gnathosoma extends medially from the apex of the ventral gnathosoma and ends blindly near the base of a single pair of hypostomatic setae (AHS, Figure 2.50). The hypostome of the larval gnathosoma appears narrower than the hypostome of other developmental stages. The subcapitular groove of the larval gnathosoma appears less developed and the subcapitular denticles are absent.

Deuteronymph gnathosoma Protonymph, deuteronymph and adult female gnathosoma are morphologically similar; therefore, this description of the deuteronymph gnathosoma applies to the gnathosoma of the other two stages. The ventral surface of the hypostome is rounded at the base and possesses two pairs of posterior hypostomatic setae (PHS, Figure 2.51). The subcapitular groove is well-defined and extends from the apex of the gnathosoma toward the

gnathosomatic base. The subcapitular groove of the deuteronymph (and all other stages) is often covered by the tritosternum, a pointed structure that arises from the gnathosomatic base and extends toward the anterior end of the gnathosoma (see Figure 2.13). Subcapitular denticles (SCD) are well-defined within the subcapitular groove on the gnathosomatic base; the subcapitular groove divides the anterior portion of the gnathosoma into two pointed structures called corniculi (CN) (sing. corniculus). The internal malae (IM) appear as a pair of pointed structures with serrate outer edges and are located between the corniculi. A pair of salivary styli (STL) are present, one on the outer side of each corniculi.

Adult male gnathosoma The male gnathosoma is distinct from the gnathosoma of all other life stages. The subcapitular groove is wide and the internal malae are fully exposed (Figure 2.52), whereas the lateral surfaces of the internal malae of all other developmental stages are almost completely covered by the hypostome when viewed from the ventral surface. Each corniculus is reduced to a narrow, sharpened point. Salivary styli are located on either side of the internal malae, between the internal malae and corniculi. Subcapitular denticles are well-defined and located within the subcapitular groove on the base of the hypostome. The spermatodactyl arms and pad-like apices (SD) are visible from the ventral surface when these are extended over the gnathosoma.

Adult female gnathosoma From the ventral surface, the female gnathosoma is similar in appearance to that of the deuteronymph (Figure 2.53) except for the position of the two pairs of hypostomatic setae. The posterior hypostomatic setae of the adult female gnathosoma appear to be almost parallel in their arrangement, whereas the innermost pair of posterior hypostomatic setae of the deuteronymph gnathosoma are positioned more anterior than the outermost pair (Figure 2.51).

Internal anatomy

Gnathosoma Scanning electron microscopy reveals the gnathosoma to consist of a pair of 5-segmented pedipalps, a pair of chelicerae and the subcapitulum; the latter consists of the hypostome which narrows anteriorly to form a pair of pointed corniculi (see Figures 2.50 to 2.53). A pair of internal malae and a pair of salivary styli are positioned between the corniculi.

A frontal section through the anterior gnathosoma shows the position of the labrum (LB), salivary styli and corniculi (Figure 2.54). Transverse pharyngeal constrictors appear in regular intervals across the pharynx (Figure 2.55, arrowheads). The pharynx (PX) extends from the anterior gnathosoma through the salivary glands (SG) and toward the synganglion (Figure 2.56). The pharynx leads to the oesophagus which passes through the synganglion (Figure 2.57) and divides it into the dorsal supraoesophageal ganglion and ventral suboesophageal ganglion. No clear boundary was observed between the pharynx and oesophagus.

A cross section through the gnathosoma (Figure 2.58) reveals the arrangement of gnathosomal, cheliceral and pharyngeal muscles and the location of the salivary glands. In cross section, the pharynx appears as a three-sided, Y-shaped structure on the ventral hypostome to which several muscles are attached (Figures 2.58 and 2.59) including the dorsal (DP) and ventrolateral (VP) pharyngeal constrictors that surround the pharynx. The dorsal (DPH) and dorsolateral (DDP) dilators of the pharynx extend from the pharynx to the ventral surface of the cheliceral sheath, and from the pharynx to the lateral wall of the hypostome, respectively. The ventrolateral dilators (VDP) extend from the lateral sides of the pharynx toward the ventrolateral surface of the hypostome. The cheliceral muscles consist of depressors (extensors) (DCH) and levators (flexors) (LCH) of the mobile cheliceral finger. The epipharyngeal levators (LEP) are ventral to the chelicerae and dorsal to the pharynx.

Synganglion The synganglion occupies the ventral region of the anterior podosoma (Figures 2.60 and 2.61) and consists of a central neuropile (NP) and peripheral cortex (CTX, Figure 2.62); the latter extends from the gnathosomatic base and ends near the genital orifice of the genital shield. The neuropile is fibrous in appearance whereas the cortex consists of rounded, darkened cells (Figure 2.63).

Digestive and excretory system The oesophagus leads to the ventriculus (V). Anterior and posterior caecae (C) extend from the central ventriculus; one pair extend anteriorly toward the gnathosoma and the other posteriorly toward the distal opisthosoma (Figures 2.64 and 2.65). The caecal wall is one cell layer thick

and microvilli (MV) form a diffuse brush border projecting into the caecal lumen (Figure 2.66). A single layer of cells form the wall of the Malpighian tubules; these cells also exhibit a diffuse border of microvilli (Figure 2.67). Cells lining the Malpighian tubules are arranged in a small ring and prominent septate-like divisions appear within these cells. Each septum extends from the basal portion of the cell toward the microvilli border and ends with a small loop (see Figures 2.67, 5.30 and 5.30 inset). Cells of the caecal wall do not have these characteristics.

Numerous cells, herein described as digestive cells (D), are observed within two masses along the mid-lateral sides of the body on either side of the ventriculus (Figure 2.68). These cells stain deeply and each has a prominent, darkened nucleus (light microscopy). The cytoplasm of these cells is dense and contains numerous vesicles when examined by TEM (Figure 2.69). Masses of these cells extend anteriorly as far as the posterior synganglion and posteriorly into the latter two-thirds of the body (Figure 2.64). The colon and rectum are located posterior to the ventriculus in the distal opisthosoma and the colon leads to the anal atrium.

Reproductive system A cross section through the genital shield shows the genital orifice and underlying genital atrium (GA) (Figure 2.70). The genital orifice, observed underlying the anterior region of the genital shield (Figures 2.41 and 2.42), extends from either side of the broad shield base toward the interior of the body to the seminal receptacle and uterus. The uterus appears as a group of invaginations which is not always distinguishable from the more ventral seminal receptacle. A single medial ovary is dorsal to the uterus (Figure 2.71). Developing eggs are observed within sac-like structures that project outward from the central portion of the ovary. Most are small, forming a cluster around the periphery of the ovary. One is prominent within the body and often occupies one-third or more of the body of a gravid female. The large developing egg (DE, Figure 2.72) contains large numbers of darkened spheres and vacuole-like structures (Figures 2.72 and 2.73). A parasagittal section through a gravid *P. persimilis* female shows the position of the reproductive system (OV, UT, SR)

with respect to the synganglion and portions of the digestive system (Figures 2.74 and 2.75).

Numerous isolated cells with prominent nuclei were observed along the dorsal and lateral sides of the body and at the tip of the opisthosoma. These cells stain deeply and occasionally appear to have a prominent nucleus when examined by light microscopy (Figure 2.76). The cytoplasm of these cells is granular in appearance (TEM) and were observed proximal to (or directly underneath) the cuticle (Figures 2.76 and 2.77). A cross section and diagram through coxae II shows the relative position of the synganglion (SN), digestive cells, caecae and opisthosomal muscles (SE, CD/TA) (Figures 2.78 and 2.79). A cross section and diagram through the genital shield and coxae IV shows the relative position of the prominent developing egg, digestive cells, caecae and Malpighian tubules (MT) (Figures 2.80 and 2.81).

Discussion

External morphology

Egg Eggs are oval and measurements (219.23±2.73 x 178±1.48 μm ; n=10) are slightly larger than those reported in an earlier study by Hessein (202 x 177 μm; Hessein, 1976). The striated pattern of older eggs observed in this study is likely the outer surface of the developing larva when viewed through the transparent chorion.

Larva Larvae are oval in shape and body measurements (233.06±7.86 x 141.02±4.77 μm; n=5) differed from those reported in a previous study. Larval body measurements reported by Hessein (1976) suggest that larvae in his study were more rounded than oval (221 x 205 μm). Hessein did not elaborate on his methods; however, illustrations included in his study suggest that his observations were acquired by light microscopy (see Appendix 5). Nor did Hessein specify landmark sites for the acquisition of body measurements taken during his study. In my study, body measurements were taken from scanning electron micrographs: width measurements taken at the widest part of the idiosoma and length measurements from the anterior gnathosoma to the tip of the distal opisthosoma. Although pedipalps are considered a part of the gnathosoma (Evans, 1992),

pedipalps were often partially extended or fully withdrawn when individuals were examined and therefore were not included in body length measurements.

Ten pairs of dorsal setae were observed on *P. persimilis* larvae and the number, size and arrangement of dorsal setae are consistent with those reported by Hessein (1976). A slightly sclerotized dorsal shield was observed with the scanning electron microscope. The dorsal shield appears as a slightly elevated ridge that extends from the first pair of dorsal setae toward the posterior opisthosoma and surrounds the ninth pair of dorsal setae. Only the posterior region of the dorsal shield is conspicuous when observed by light microscopy. According to Hessein (1976), the dorsal shield is located on the posterior opisthosoma, located between the ninth and tenth pair of dorsal setae. This represents only a small portion of the entire shield and corresponds to the posterior region of the dorsal shield illustrated in Figure 2.2.

Hessein observed larvae with both three and four pairs of preanal setae and he considered those with three pairs as male, whereas those with four pairs were considered female. No such observations were made in my study although this distinction between male and female could have been overlooked due to the small number of specimens examined during this study.

Ventral shields were not observed on *P. persimilis* larvae and this observation is consistent with those made by Hessein (1976). Two previously unreported pores adjacent to the paranal setae were observed in all immature stages; however, their function is not known. The cribrum, a spiculated area posterior to the anus, was present in all other stages but absent from the larval stage.

Protonymph Protonymph body measurements (258.44±7.64 x 143.04±5.98 μm; n=5) were smaller than those reported in a previous study (269 x 221 μm; Hessein, 1976). The dorsal shield is lightly sclerotized with chaetotaxy similar to the adult female. Chaetotaxy is in agreement with observations reported by Hessein (1976). Although *P. persimilis* protonymphs were smaller than deuteronymphs, the lightly sclerotized dorsal shield and arrangement of dorsal setae were similar to those observed on deuteronymphs of *P. persimilis*. A short

peritreme was observed on *P. persimilis* protonymphs during my study and this observation is consistent with those reported by Hessein (1976).

Although ventral shields are absent, the anus is located on a smooth surface on the distal opisthosoma that could be described as a poorly-defined anal shield. Ventral setae include three pairs of sternal setae (on the podosoma), three pairs of preanal setae (on the opisthosoma), a pair of paranal setae and a single postanal seta (distal opisthosoma, adjacent to anus). These observations were in general agreement with those of Hessein (1976). However, his observation that male protonymphs have three pairs of preanal setae and female protonymphs four pairs was inconsistent with my observations. It is possible that this distinction was not made during my study due to the small number of individuals examined.

Deuteronymph Deuteronymph body measurements (293.83±6.59 x 161.83±7.89 μm; n=3) are smaller than those reported in a previous study (300-348 x 237-269 μm; Hessein, 1976). Sixteen pairs of dorsal setae were observed on a lightly sclerotized dorsal shield and the number, size and arrangement of dorsal setae are consistent with those reported by Hessein (1976). Chaetotaxy is similar to that of the adult female.

Deuteronymph chelicerae are well developed. According to Evans (1992), the role of the trichoid sensilla on the fixed digit of the chelicerae is unknown in the Acari. It is believed, however, that the pilus dentilis could have a mechanoreceptive function, whereas the apical pit sensillum is thought to have a gustatory function. There was no evidence of pores in these sensilla. Dorsal and lateral slit organs measure strain induced by muscular activity, haemolymph pressure and substrate vibrations (Evans, 1992). These sensilla were observed on chelicerae of deuteronymphs and adults (chelicerae of larvae and protonymphs were not observed by SEM).

The subcapitulum, or ventral gnathosoma, of the Acari is formed by enlarged coxae of the pedipalps which meet and fuse on the ventral surface of the gnathosoma (Evans, 1992). The subcapitular groove acts as a channel which conducts food forward and denticles found in this groove may prevent large food

particles from being ingested by the predator. The bifurcate tritosternum is thought to work together with other gnathosomal processes to assist with the redirection of prey fluids from the presternal region toward the hypostome (Wernz and Krantz, 1976).

Conspicuous ventral shields (sternal or anal) are not present. When examined by SEM, a smooth surface surrounds the anus and this could be described as a poorly-defined anal shield. Hessein (1976) reported male deuteronymphs to be smaller in size than female deuteronymphs; the ventral setae of male deuteronymphs are similar in arrangement to those of adult males, whereas the ventral setae of female deuteronymphs are similar to those of adult females. Although no such observations were made during my study, these features could have been overlooked due to the small number of individual deuteronymphs examined.

A slightly sculptured surface on the posterior opisthosoma extends from the most posterior pair of sternal setae toward and around the anus, paranal setae and post anal seta. Although a sclerotized anal shield was not observed, a smooth, non-sculptured area surrounding the anus could be considered analogous to an anal shield and is visible by light microscopy. The cribrum, a spiculated area posterior to the anus, is observed with both light and scanning electron microscopy.

Adult male Body measurements of *P. persimilis* adult males (323.60±16.82 x 188.28±2.42 μm; n=4) differed from those reported in a previous study (300 x 237 μm; Hessein, 1976). Although the dorsal shield was less pronounced than that of the adult female, chaetotaxy of the 16 pairs of dorsal setae was similar to that of the adult female and in agreement with observations made by Hessein (1976).

The dentate ventral surface of the fixed digit of male chelicerae is more prominent than in those of other developmental stages and the upward angle of the curve of the single tooth-like spine on the moveable digit is more conspicuous. These morphological features of the adult male cheliceral digits may be modifications for their specialised use during mating. The more prominent dentation could be used to grasp females during mating or to help facilitate

spermatophore transfer. The number and relative placement of trichoid sensilla (mechanoreceptors) and slit organs are similar to those observed on deuteronymph chelicerae. The apical pit sensillum is likely to have a gustatory function (Evans, 1992).

In a previous study (Wainstein, 1973) a diagram of the chelicerae of a male phytoseiid includes the medial pilus dentilis; however, the dorsal and lateral slit organs and apical pit sensillum were not illustrated (Figure 2.82). The moveable digit shows a dentate surface but the prominent tooth-like spine is absent. It is not known if these morphological differences are unique to *P. persimilis* or are general features of the genus.

A diagram of the spermatodactyl of a phytoseiid male (Figure 2.82), as illustrated by Wainstein (1973), bears an overall similarity to the spermatodactyl of *P. persimilis* males observed in this study (Figure 2.83). The diagram shows the broad base of the spermatodactyl arm extending ventrally to form a single, broad arm called the truncus (t). Two rounded projections on the truncus, referred to as the velum (v) (proximal) and lamellum (l) (distal) project toward the anterior of the spermatodactyl arm. The velum extends from the spermatodactyl base and is directed anteriorly toward the medial portion of the spermatodactyl arm. Due to the length of this structure, it may be interpreted as an outward flap-like extension of the spermatodactyl arm. In *P. persimilis*, the broad base of the spermatodactyl extends anteriorly at a downward angle to become the spermatodactyl arm (Figures 2.21 and 2.83). A suture on the inside of the spermatodactyl arm divides the arm into two regions; the outer spermatodactyl arm terminates with a horn-like projection and the interior arm continues to extend at a downward angle and terminates with the broad spermatodactyl apex. A second horn-like projection is located medially on the inner spermatodactyl arm. The velum of the spermatodactyl (Wainstein, 1973) appears similar to what was described herein as the outer partition of the spermatodactyl arm of *P. persimilis* males. There is no clear reference to a suture on the inner side of the spermatodactyl arm giving the arm the appearance that it is partitioned into two regions. The lamellum is depicted with a rounded surface and it appears to be

located in a similar position as the horn-like projection on the inner partition of the spermatodactyl arm of *P. persimilis* adult males. There is no structure that compares to the second horn-like structure on the outer partition of the spermatodactyl arm.

Wainstein (1973) depicted the spermatodactyl apex as a flattened base that broadens symmetrically at the distal apex (process apicalis, ap), the apex extending from both sides of the spermatodactyl arm. An internal canal (canalis, c) is shown to extend from the spermatodactyl base and to terminate as an open pore on the broadened spermatodactyl apex. Although the observation of a pore on the spermatodactyl apex of *P. persimilis* males is consistent with observations by Wainstein (1973), the spermatodactyl apex of *P. persimilis* broadens from only one side. The resulting apical pad is more asymmetrical than the one depicted by Wainstein (1976).

The shape and position of the ventral shields (both sternogenital and ventro-anal), and arrangement of ventral setae, are consistent with those reported previously by Hessein (1976; Appendix 5). He included an unidentified structure on the anterior region of the sternogenital shield which may be a representation of the male genital orifice.

Adult female Body measurements of *P. persimilis* adult females (468.04±4.09 x 293.44±4.56 μm; n=5) were larger than those reported in an earlier study (363 x 284 μm; Hessein, 1976). The number and arrangement of dorsal setae agree with the descriptions of Hessein (1976) and Chant (1959). Hessein (1976) reported a slight serration of all dorsal setae occurs in all developmental stages, with the fourth pair of lateral setae being comparatively more serrate. In my study, the larger dorsal setae of all *P. persimilis* developmental stages are bristled, with exception of those of the larvae whose larger setae are smooth. The surface of the shorter dorsal setae are smooth. The function of the two elevated dome-like structures located on the anterior holodorsal shield is unknown. The reticulate pattern of the holodorsal shield was reported earlier by Hessein (1976).

Stigmata of *P. persimilis* adult females are located on the podosomatic

region dorsolateral to coxae of legs III and IV. The position of the stigmata is in agreement with those described on other mesostigmatid mites (Evans, 1992). The function of the peritreme and micropapillae is unclear; the latter may prevent entry of foreign particles or water into the stigmata and underlying tracheae.

The shape of the sternal shield and chaetotaxy are consistent with observations reported by Hessein (1976). In his illustration of the ventral surface of the adult female, a broad, shield-shaped genital shield extends both anteriorly and laterally to the two metasternal setae. The position of the genital orifice was not depicted. The genital shields of *P. persimilis* females in my study are pear-shaped with prominent sulcate ridging on the lateral and posterior edges. The genital orifice, located near the anterior region of the genital shield, is clearly visible (SEM).

The cribrum, a spiculated area posterior to the anus, is observed on all developmental stages excluding the larvae. The cribrum is believed to provide a dispersal platform for sex pheromones produced by proximal cribral glands (Evans, 1992).

Legs Leg morphology is consistent with previous descriptions (Evans, 1992). Setae on the dorsal surface of the tarsi are thought to have an olfactory function (Evans, 1992). The terminal podomere, or apotele, is thought to be primitive and some researchers have suggested that it is represented in extant species only by the claw complex, consisting of a basilar piece and claws (see Evans, 1992). The pulvillus, located at the apex of the ambulacrum is most developed in free-living mesostigmatid mites and can be used to grasp a surface by means of suction (Evans, 1992). *Phytoseiulus persimilis* were able to walk on webbing produced by prey mites by grasping individual strands of webbing with their claws and the scale-like processes of the ambulacrum.

The gnathosoma

Pedipalps appeared to be similar among all developmental stages of *P. persimilis*; podomeres and setae were readily identified and labelled based on illustrations from previous studies (see Evans, 1992). Although numerous terminal sensilla are located on the tips of the tarsi, these were not examined in

detail. Jagers op Akkerhuis *et al.* (1985) reported the terminal sensilla of *P. persimilis* tarsi as highly innervated and similar in appearance to contact chemoreceptors in ticks, (Ivanov and Leonovich, 1979; as cited by Evans, 1992) insects and spiders. Numerous pores in the walls of dorsal setae are consistent with a chemosensory or thermosensory role as described from insects and ticks (Jagers op Akkerhuis *et al.*, 1985). Gustatory receptors are chiefly associated with the gnathosoma of mites, especially the tarsi of the pedipalps (Evans, 1992).

The larval gnathosoma is distinct from the gnathosoma of other life stages; the former has a lesser developed subcapitular groove extending medially from the apex of the ventral gnathosoma and ending blindly near the base of a single pair of hypostomatic setae (Figure 2.49). Subcapitular denticles are absent from the subcapitular groove and the base of the hypostome is narrow. The incompletely developed gnathosoma of *P. persimilis* larvae support the observation that the larvae do not feed (Laing, 1968).

The ventral surface of the protonymph hypostome is almost identical to that of the deuteronymph and adult female. Gnathosoma of all post-larval stages have two pairs of posterior hypostomatic setae and a well-defined subcapitular groove that contains anteriorly-directed denticles.

The ventral gnathosoma of the adult male is distinct from the gnathosoma of other stages. The anterior region of the subcapitular groove is wider than in other stages, the hypostome and corniculi are reduced and the internal malae are fully exposed. The salivary styli of the adult male are located on either side of the internal malae, between the internal malae and corniculi, whereas the salivary styli of all other developmental stages are positioned on the external side of the corniculi. These modifications may contribute toward increased flexibility which may be necessary to permit full extension of the chelicerae and spermatodactyl. Because the internal malae of all other life stages were almost completely covered by the hypostome when viewed from below, it was not possible to determine the morphology of these structures.

Internal anatomy

Gnathosoma The components of the gnathosoma of the Acari function

collectively as a sensory structure used for prey capture and consumption (Chant, 1985). Setae on the tips of the pedipalps of *P. persimilis* lack porous walls which suggest that they function as mechanoreceptors (Jagers op Akkerhuis *et al.*, 1985). Chelicerae are likely used to grasp and detain prey while the sharp corniculi pierce the body to initiate feeding. Malae are thought to be of setal origin and to assist in extracting body fluids (Evans, 1992).

Frontal and cross sections through the gnathosoma show numerous gnathosomal, cheliceral and pharyngeal muscles. Transverse dilator and constrictor muscles alternate along the length of the pharynx (Evans, 1992). Attachment sites of these muscles, and muscles of the legs and opisthosoma, were reported by Akimov and Yastrebtsov (1987). The pharynx has been described as a heavily muscled suction pump (Chant, 1985) and observations here are consistent with those reported in earlier studies (Starovir, 1973; Akimov and Yastrebtsov, 1987).

The salivary glands of *P. persimilis* were observed on either side of the pharynx within the gnathosoma and are consistent with those illustrated by Starovir (1973). Although Akimov and Starovir (1978) reported one dorsal, one ventral, and two cheliceral salivary glands, it is not clear how these are distinguished or where each of these are located. Only one pair of salivary glands were observed during my study and these were located in the gnathosoma on either side of the pharynx. The salivary glands are well developed (Akimov and Starovir, 1978) and lead to the salivary styli (Evans, 1992). It is believed that salivary enzymes are injected into prey to facilitate predigestion of body contents (Starovir, 1973). Salivary gland cells and alveoli degenerate as secretions are released (Akimov and Starovir, 1978).

Digestive and excretory systems According to Evans (1992), the alimentary canal of mesostigmatid mites consists of the pharynx, oesophagus, ventriculus (mid-gut), colon, rectum and anal atrium. Although Akimov and Starovir (1978) speculated that muscles are present in the oesophagus of *P. persimilis* which aid in swallowing, another report suggested that they are absent (Starovir, 1973; as cited by Chant, 1985). The oesophagus passes through the synganglion along its central

axis and divides it into the dorsal supraoesophageal ganglion and ventral suboesophageal ganglion, a characteristic of all Acari (Krantz, 1978).

Two pairs of diverticula are present in *P. persimilis*; one pair extends toward the anterior, the other toward the posterior. Based on illustrations by Starovir (1973) and Akimov and Starovir (1978), three pairs of diverticula extend from the ventriculus; two pairs extend toward the anterior and one toward the posterior. A second pair of anterior caecae are illustrated anterior to the ventriculus and because these appear to be considerably smaller than the other pair of anterior caecae they were likely overlooked during my study.

Microvilli were observed lining the cells of the caecae and Malpighian tubules. The caecae provide additional surface area for digestion (Akimov and Starovir, 1978). Microvilli on cells of the Malpighian tubules increase the surface area of these cells and may assist in the transport of sodium, potassium and other ions across the walls of these cells.

Digestion within the ventriculus is intracellular and is undertaken by vacuolated epithelial cells which absorb soluble food and are eventually shed into the caecal lumen (Starovir, 1973; Akimov and Starovir, 1974). Starovir (1973) reported three types of cells: digestive, secretory and undifferentiated. Digestive cells reacted rapidly to influx of food but degenerated every few minutes and were replaced by undifferentiated cells (Akimov and Starovir, 1974).

Digestive cells in *P. persimilis* are depicted as rounded cells with prominent nuclei and numerous vacuoles (Starovir, 1973; Akimov and Starovir, 1974; 1978). These epithelial cells, reported within the midgut (ventriculus), lack microvilli and bear a resemblance to what are herein described as digestive cells during my examination of *P. persimilis*. According to Evans (1992), walls of the ventriculus and caecae are formed by a single layer of epithelial cells that rest on a thin basement membrane. Furthermore, Woodring and Galbraith (1976) reported that the ventriculus and caecae in the mesostigmatid mite *Fuscouropoda agitans* Banks appear morphologically identical. Based on this information, it is likely that what were described as digestive cells in my study are not analogous to descriptions of digestive cells in previous studies of *P. persimilis*.

Illustrations and description of the lyrate organ in the gamasid (mesostigmatid) mite *Haemogamasus horridus* Mitch. (Michael, 1892) bear a remarkable resemblance to cells described herein as digestive cells in *P. persimilis*. The entire structure of the lyrate organ included two flattened arms extending from either side of a globular ovary. Michael (1892) reported the lyrate organ to vary from one individual to the next while remaining similar enough among individuals to accurately predict a species based solely on the shape and appearance of this organ. The lyrate organ was one of the largest organs observed in *H. horridus*; the densely packed and clearly defined cells stain deeply, having well defined nuclei and lacking any intracellular space or tissue. The lyrate organ of the spiny rat mite, *Echinolaelaps echidninus* L. is similar in appearance to that of *H. horridus* but much smaller (Jakeman, 1961). The lyrate organ was absent from detailed illustrations of the internal anatomy of the mesostigmatid mite *Fuscouropoda agitans* (see Woodring and Galbraith, 1976). Positive identification of what are herein described as digestive cells in *P. persimilis* is further complicated by the similar appearance of these cells and diagrams of those lining the ventriculus in *F. agitans* (see Woodring and Galbraith, 1976).

Reproductive system The lyrate organ has been described in representatives from a few families of mites (Michael, 1892). Although Michael (1892) suggested that the lyrate organ could function as either a germiniferous or vitelligenic organ, the former hypothesis was favoured. Based on observations made in this and previous studies, it seems reasonable to conclude that "digestive cells" of *P. persimilis* are actually cells of the lyrate organ. The presence of numerous vesicles within these cells suggest that they may function as a vitelligenic organ and that they are involved in loading yolk into the rapidly maturing eggs.

If this proves to be the case, this may provide some insight regarding the reproductive potential of ovipositing *P. persimilis* females. Some ovipositional females are capable of producing six or more eggs per day (data not shown; mean fecundity data reported in Chapter 6) and each egg is about half the size of the adult female body (based on measurements taken during this study, Table 2.1). Examination of prominent developing eggs within gravid females showed them to

be comprised primarily of dark and vacuole-like spheres. These are believed to be yolk and lipid, respectively. Spherical structures are absent within immature eggs that develop within sac-shaped structures of the medial ovary. This suggests that yolk is rapidly loaded into eggs during development. Therefore, it seems reasonable to conclude that what Michael (1892) described as cells of the lyrate organ, and described here as "digestive cells", likely function as a vitelligenic organ and are responsible for the rapid addition of yolk to developing eggs. The size of this organ and proficiency of egg production in *P. persimilis* support this theory.

Although spermatogenesis and the male genital system of *P. persimilis* were described by Petrova (1970), and the spermatodactyl and spermatheca of phytoseiid mites by Wainstein (1973), little information is available regarding the reproductive system of adult female *P. persimilis*. Observations described here suggested that *P. persimilis* females have one medial ovary and this observation is consistent with previous reports of ovaries in other mesostigmatid mites (Michael, 1892; Jakeman, 1961; Woodring and Galbraith, 1976). The overall position of reproductive and digestive tissues appeared similar to those reported in previous studies for other mesostigmatid mites. However, further investigation is required to provide information regarding reproductive tissues and accessory glands of male *P. persimilis* and more detailed information of the female reproductive system.

Synganglion The synganglion consists of a central neuropile, comprising a large portion of the central area of the synganglion, and a peripheral cortex. The synganglion of the Acari have not been studied extensively; however, the cortical region of the synganglion of ticks consists of motor neurones, neurosecretory cells and olfactory globuli cells. The neuropile is comprised of axons and dendrites (Evans, 1992). The synganglion of *P. persimilis* occupies the ventral region of the anterior podosoma. Peripheral nerves have been described from ticks (see Evans, 1992); however information regarding the nervous system of *P. persimilis* and other Acari is lacking. Further histological study is required to describe details of the nervous system.

Cells underlying the cuticle Epidermal cells in the mesostigmatid mites

Fuscouropoda agitans (see Woodring and Galbraith, 1976) and *Haemogamasus horridus* (see Michael, 1892) are depicted to underlie the cuticle and extend from the anterior-most region to the posterior opisthosoma. In *P. persimilis*, cells with prominent nuclei directly underlie (or are proximal to) the cuticle. These cells do not appear to extend from the anterior-most region of the body to the distal opisthosoma. Although the function of these cells is unknown, they appear morphologically similar to oenocytes and may have a similar function.

Figures 2.1-2.6. Dorsal and ventral view of *Phytoseiulus persimilis* larvae.

Figure 2.1. Dorsal surface.

Figure 2.2. Composite diagram of dorsal surface showing arrangement of dorsal setae.

Figure 2.3. Ventral surface. A single pair of ventral pores (arrowheads) are located posterior to the third pair of sternal setae; both interior and posterior to the coxae of leg III.

Figure 2.4. Diagram of ventral surface showing arrangement of ventral setae.

Figure 2.5. Pore on ventral surface of larva (enlargement of ventral pore in Figure 2.3).

Figure 2.6. Larval anus showing setae and two pores, one on the outer side of each paranal seta.

Scale bars: Figures 2.1, 2.2, 2.3 and 2.4, 50 μm; Figure 2.5, 1 μm; Figure 2.6, 10 μm.

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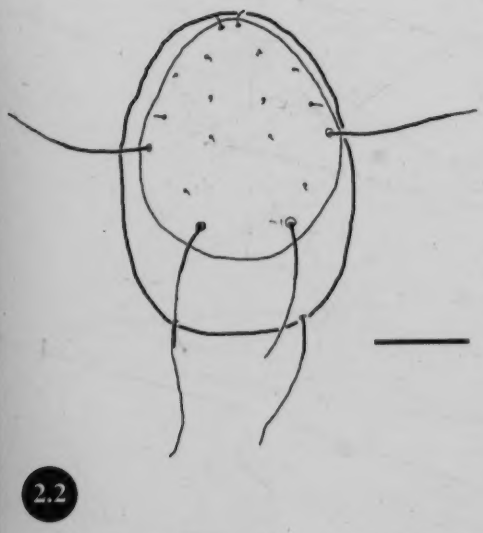
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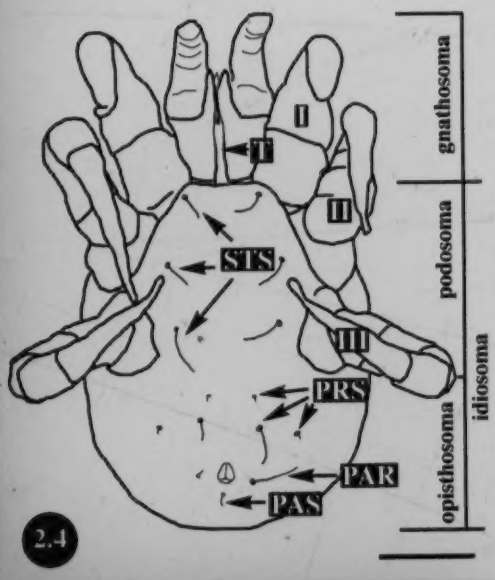
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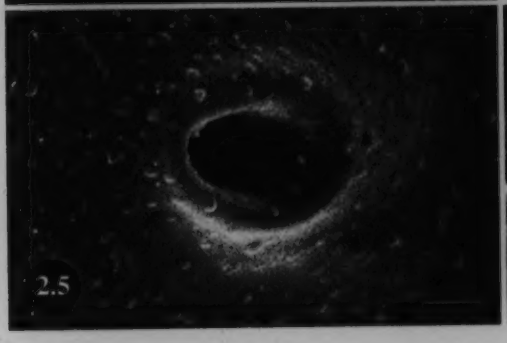
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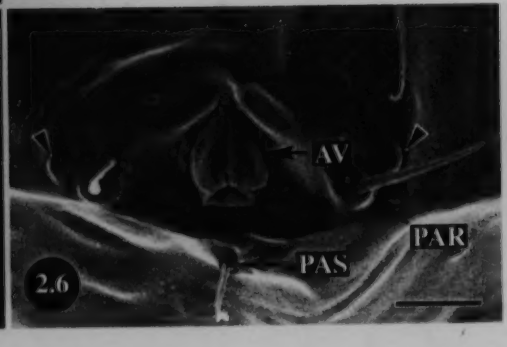
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Figures 2.7-2.12. Dorsal view and chelicerae of *P. persimilis* deuteronymphs.

Figure 2.7. Dorsal surface.
Figure 2.8. Composite diagram of dorsal surface showing dorsal shield and arrangement of dorsal setae.
Figure 2.9. Lateral view of deuteronymph chelicerae. Inset: Cheliceral claws showing the medial pilus dentilis and apical pit sensillum.
Figure 2.10. Dorsal view of chelicerae showing the teardrop-shaped trichoid sensillum on the dorsal surface (arrowhead).
Figure 2.11. Ventral view of the fully extended chelicerae.
Figure 2.12. Ventral view of hypostome, pedipalps (trochanter and femur) and partially extended chelicerae.

Scale bars: Figures 2.7 and 2.8, 50 μm; Figures 2.9 and 2.11, 5 μm; Figure 2.9 inset, 1 μm; Figures 2.10 and 2.12, 10 μm.

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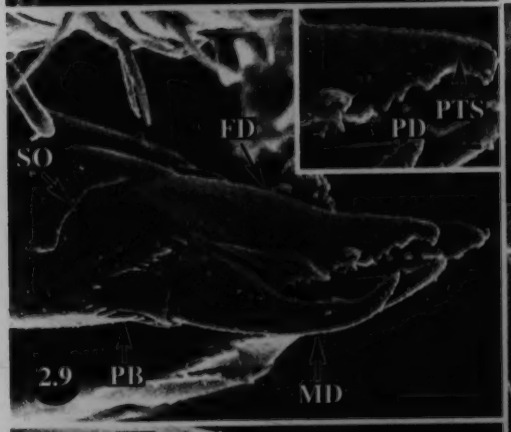
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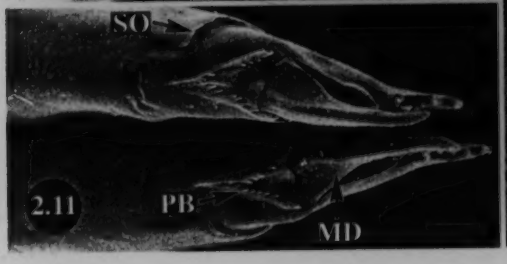
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Figures 2.13-2.16. Ventral view of *P. persimilis* deuteronymphs.

Figure 2.13. Gnathosoma showing bifurcate tritosternum and subcapitular groove; the latter extends from the anterior gnathosoma toward the gnathosomatic base. Anteriorly directed subcapitular denticles are arranged in transverse rows within the subcapitular groove. (See Figures 2.51 to 2.53 for higher magnification of denticles and other gnathosomal characteristics.)

Figure 2.14. The ventral podosomal region showing the arrangement of the five pairs of sternal setae.

Figure 2.15. Posterior opisthosoma showing three pairs of preanal setae.

Figure 2.16. Deuteronymph anus showing one pair of paranal setae, a single postanal seta, cribrum, and two pores, one on the outer side of each paranal seta.

Scale bars: Figures 2.13 and 2.15, 20 μm; Figure 2.14, 50 μm; Figure 2.16, 10 μm.

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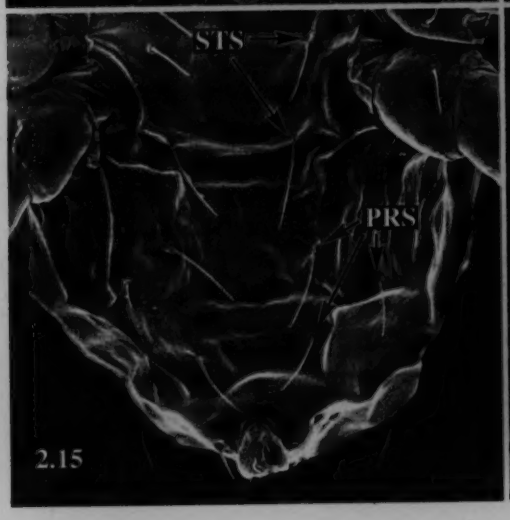
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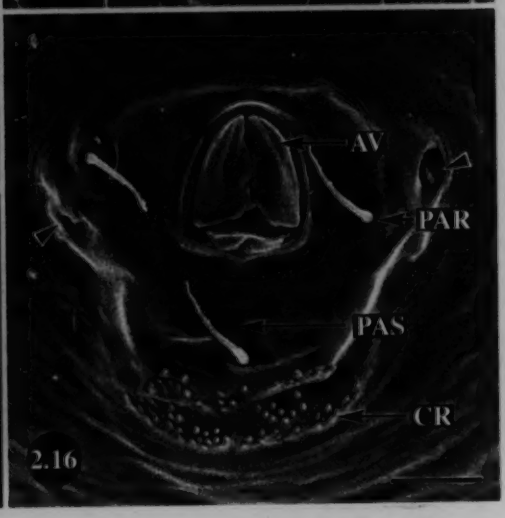
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Figures 2.17-2.22. Dorsal view and chelicerae of *P. persimilis* adult males.

Figure 2.17. Dorsal surface.

Figure 2.18. Composite diagram of dorsal surface of an adult male showing dorsal shield and arrangement of dorsal setae.

Figure 2.19. Chelicerae of male retracted into cheliceral sheath under the hood-like gnathotectal process. The moveable digit appears as an upward-curving spine-like tooth whose tip appears to fit within a depression on the outer surface of the fixed digit. Each spermatodactyl arm extends anteriorly and ends with a broad, pad-like apex.

Figure 2.20. Ventro-lateral view of spermatodactyl partially extended over hypostome.

Figure 2.21. Fully extended chelicerae of a *P. persimilis* male. Apex of spermatodactyl in foreground missing. Spermatodactyl arm appears to be partitioned into an inner and outer portion; the former extends farther anteriorly than the latter. Two trichoid sensilla on the fixed digit include the pilus dentilis and teardrop-shaped dorsal seta (arrowhead). Inset: Suture on inner surface of the spermatodactyl arm gives the appearance that the arm is partitioned into an inner and outer region. Two horn-like projections (arrowheads) are positioned at the junction of the inner and outer spermatodactyl arm.

Figure 2.22. Broad apex of spermatodactyl; the pore in the apex is covered with debris. Two horn-like projections extend from the inner (left arrowhead) and outer (right arrowhead) regions of the spermatodactyl arm. Inset: Pore (arrowhead) in broad spermatodactyl apex.

Scale bars: Figures 2.17 and 2.18, 50 μm; Figures 2.19 and 2.20, 5 μm; Figure 2.21, 10 μm; Figures 2.21 inset, 2.22 and 2.22 inset, 1 μm.

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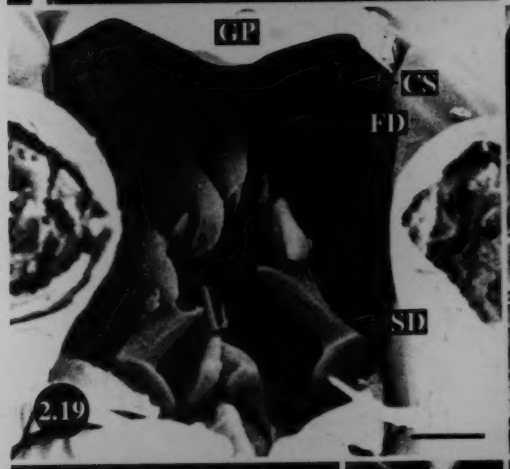
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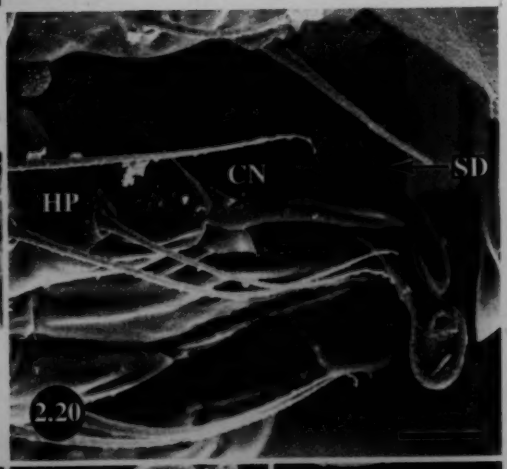
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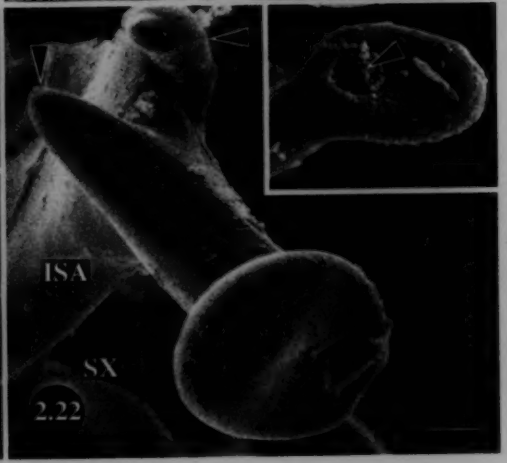
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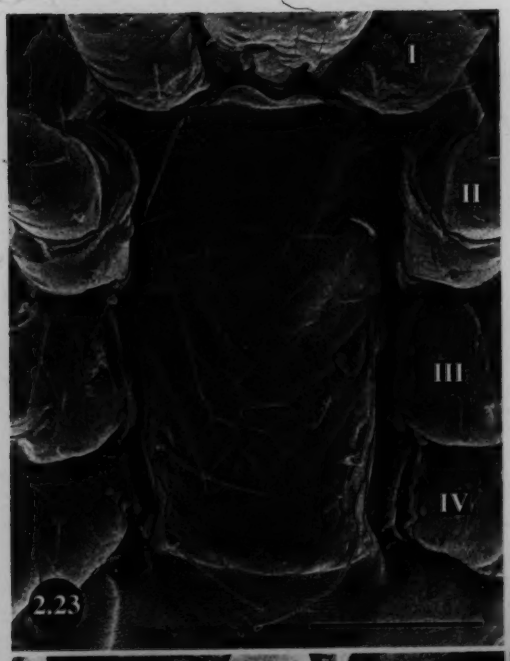
Figures 2.23-2.28. Ventral view of *P. persimilis* adult males.

Figure 2.23. Sternogenital shield showing reticulate surface sculpturing.
Figure 2.24. Diagram of sternogenital shield showing the position of the five pairs of sternal setae and male genital orifice.
Figure 2.25. Genital orifice of an adult male.
Figure 2.26. Two unidentified structures (arrowheads) that protrude from the genital orifice of a *P. persimilis* adult male may be part of the male reproductive system or a portion of a spermatophore.
Figure 2.27. Ventro-anal shield showing reticulate surface sculpturing.
Figure 2.28. Diagram of ventro-anal shield and arrangement of ventral setae on the distal opisthosoma.

Scale bars: Figures 2.23, 2.24, 2.27 and 2.28, 50 μm; Figures 2.25 and 2.26, 5 μm.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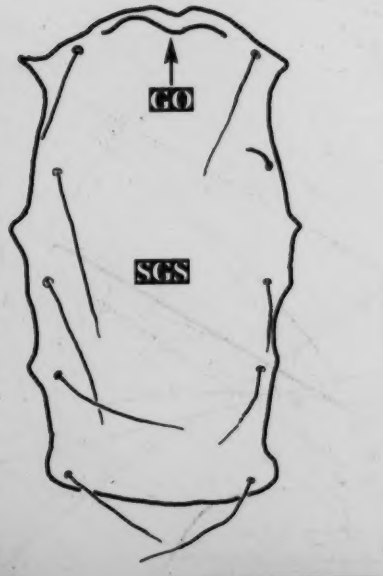
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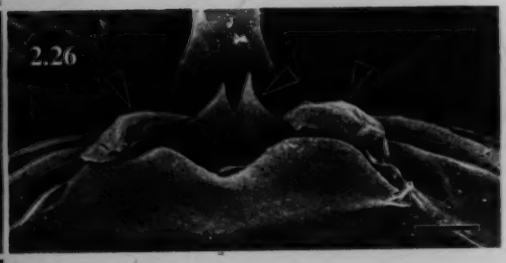
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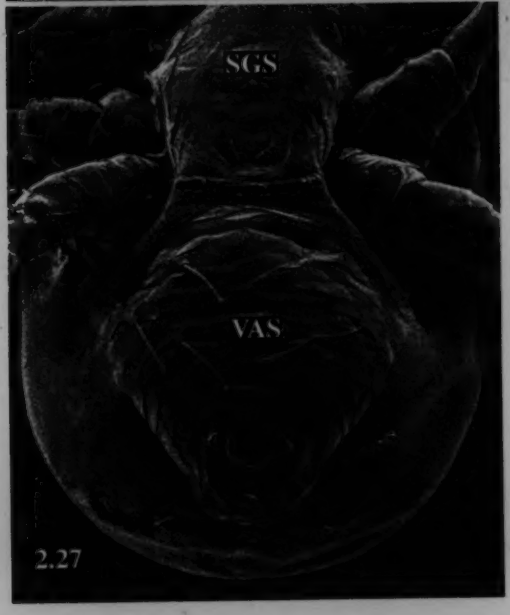
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Figures 2.29-2.34. Dorsal view and chelicerae of *P. persimilis* adult females.

Figure 2.29. Dorsal surface.

Figure 2.30. Composite diagram of dorsal shield and arrangement of setae of an adult female.

Figure 2.31. The fourth anterior pair of dorsal setae with bristled surfaces. Inset: Two dome-shaped structures on dorsal shield, located posterior to the most anterior pair of dorsal setae and between the second anterior pair of dorsal setae.

Figure 2.32. Stigma positioned dorsolaterally between coxae of legs III and IV. The peritreme, an anteriorly directed groove, is associated with each stigma and extends from the stigma toward the dorsolateral side of leg II. Inset: Tuberculate micropapillae within the peritreme.

Figure 2.33. Ventro-lateral view of gnathosoma, chelicerae and pedipalps (trochanter, femur) of an adult female.

Figure 2.34. Chelicera of an adult female.

Scale bars: Figures 2.29 and 2.30, 100 μm; Figures 2.31, 2.31 inset and 2.33, 10μm; Figure 2.32, 50 μm; Figure 2.32 inset, 1 μm; Figure 2.34, 5μm.

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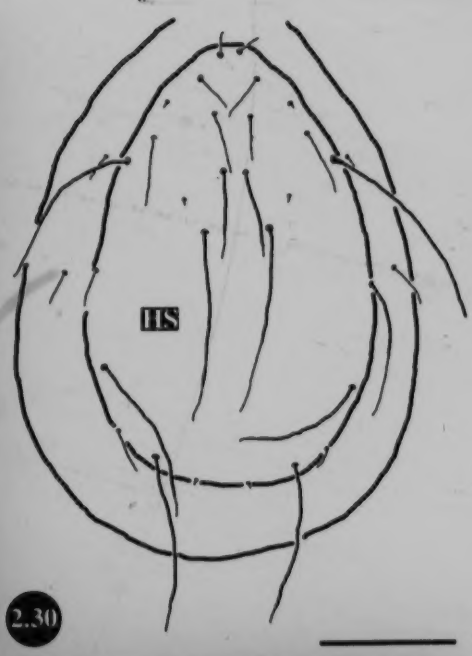
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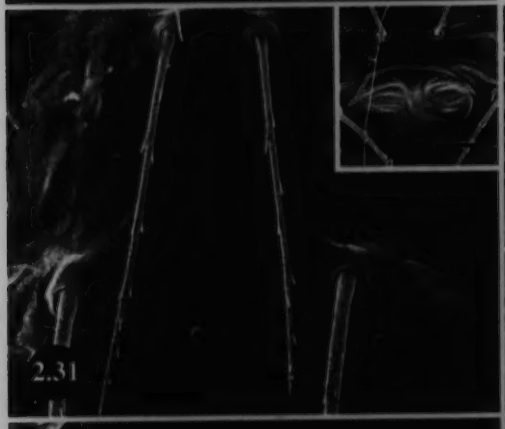
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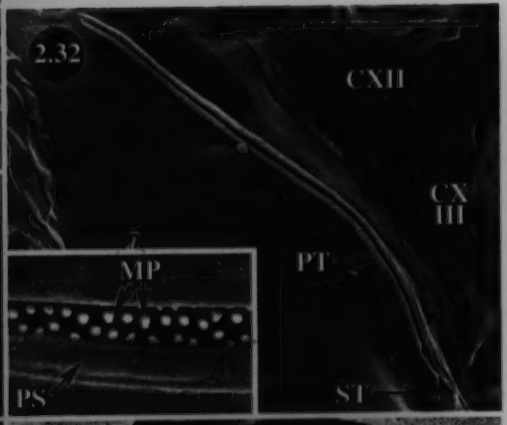
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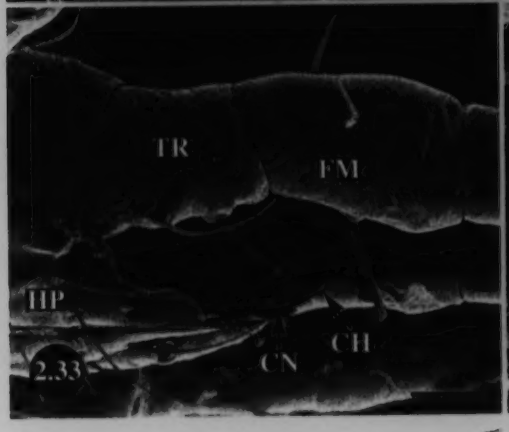
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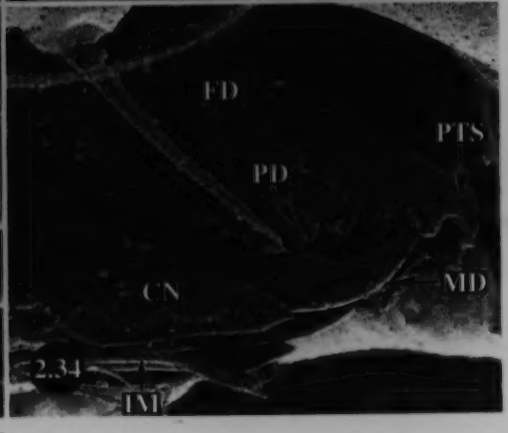
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Figures 2.35-2.40. Dorsolateral and ventral views of *P. persimilis* adult females.

Figure 2.35. Dorsolateral view of an adult female showing overall position of stigma and peritreme and reticulate surface sculpturing of holodorsal shield. The metapodal shield (arrowheads) is located on the dorsolateral surface posterior to coxae of leg IV.

Figure 2.36. Metapodal shield.

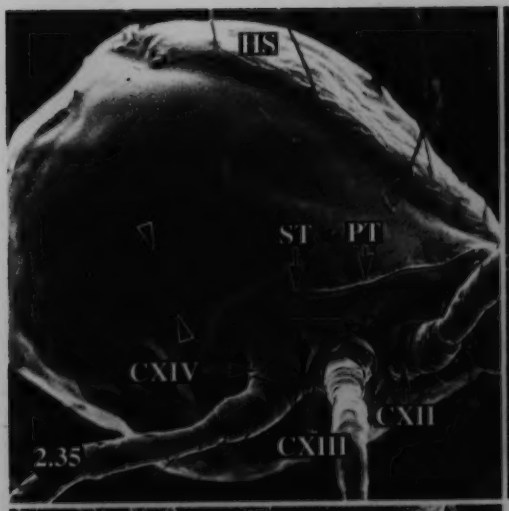
Figure 2.37. Sternal shield showing the reticulate pattern on its surface.

Figure 2.38. Diagram of sternal shield and arrangement of three pairs of sternal setae.

Figure 2.39. Genital shield of adult female.

Figure 2.40. Diagram of the pear-shaped genital shield and arrangement of setae. Dashed line represents the approximate location of the underlying female genital orifice.

Scale bars: Figures 2.35, 2.37, 2.38, 2.39 and 2.40, 50 μm; Figure 2.36, 10 μm.



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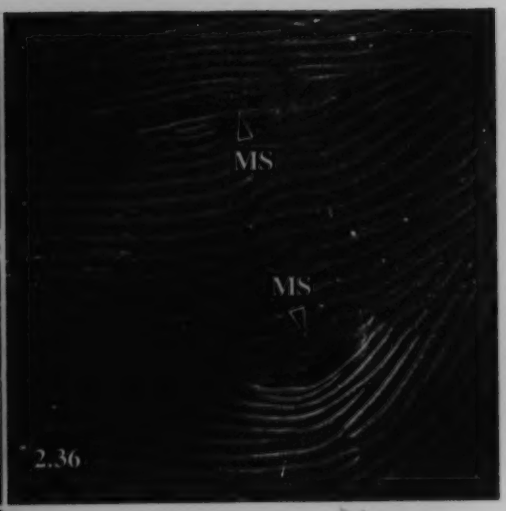

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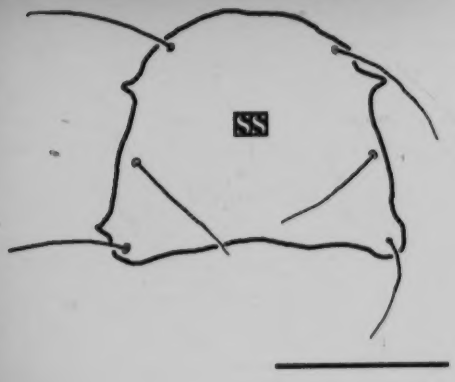

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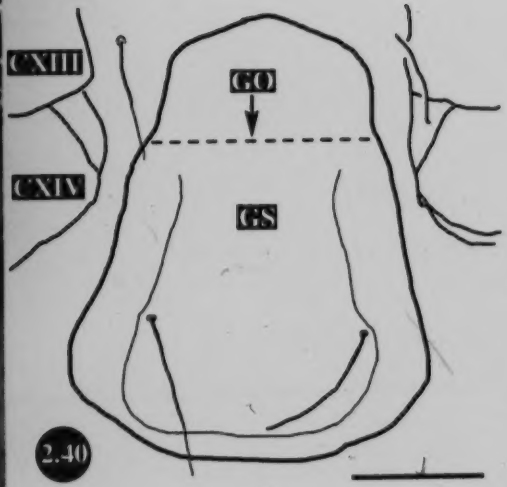


2.40

Figures 2.41-2.44. Ventral view of *P. persimilis* adult females.

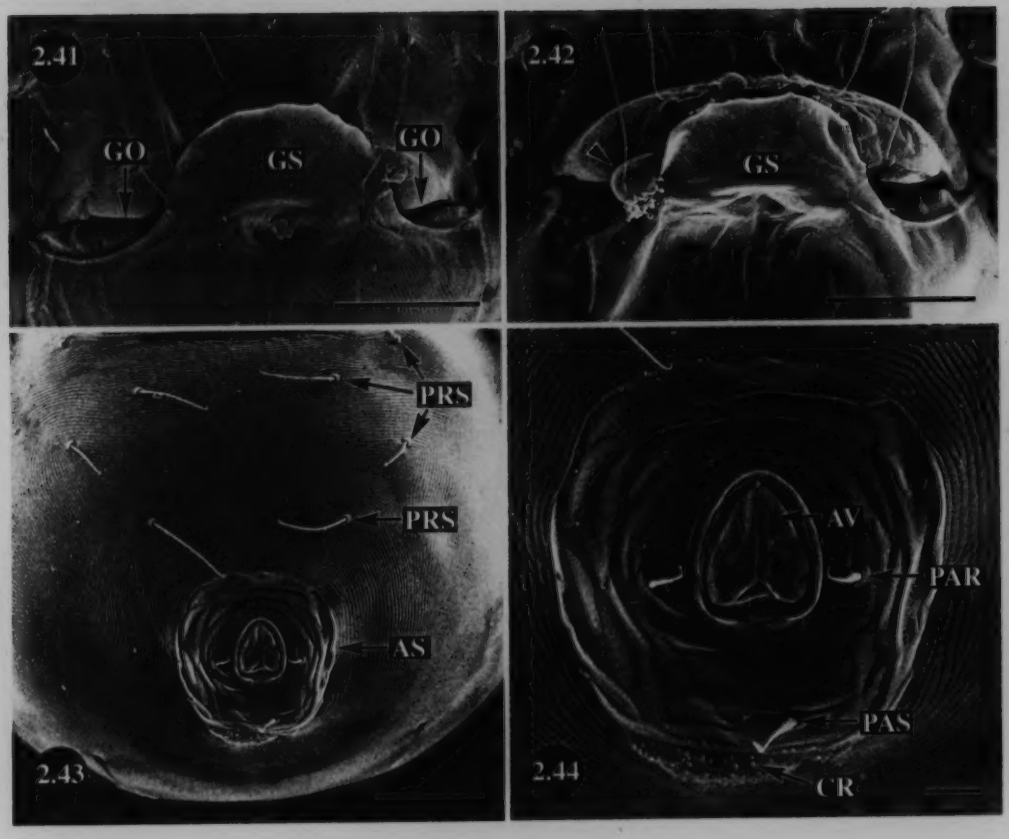
Figure 2.41. Genital orifice of an adult female partially concealed by the anterior region of the genital shield.

Figure 2.42. Possible spermatophore (arrow) within the genital orifice of an adult female. The area directly above the genital shield is considerably different than the same area shown in Figure 2.41. It is not known if both females had been inseminated.

Figure 2.43. Posterior opisthosoma of an adult female showing arrangement of opisthosomal setae and position of anal shield.

Figure 2.44. Anus and associated anal setae positioned on anal shield.

Scale bars: Figures 2.41, 2.42 and 2.43, 50 μm; Figure 2.44, 10 μm.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Figures 2.45-2.47. Detail of *P. persimilis* legs.

Figure 2.45. Ventro-lateral surface of leg III (deuteronymph).

Figure 2.46. The ambulacrum (elongated distal apex) of the tarsus of an adult male. A lateral dentate process (arrowhead) arises from the base of the ambulacral stalk; the stalk is covered in deeply ridged and elongated processes. Inset: Apex of ambulacrum showing pulvillus and one of two claws (arrowhead).

Figure 2.47. Ventral view of a deuteronymph walking on webbing made by the prey mite, *Tetranychus urticae*. The round sphere (upper left) is a *T. urticae* egg. Inset: Apex of the ambulacrum showing the pulvillus and claw. Elongated scale-like structures on the ambulacral stalk (upper arrowhead) and claws (lower arrowhead) are used to entangle and grasp prey webbing (cryo SEM).

Scale bars: Figure 2.45, 20 μm; Figure 2.46, 10 μm; Figure 2.46 inset, 5 μm; Figure 2.47, 100 μm; Figure 2.47 inset, 2 μm.

2.45

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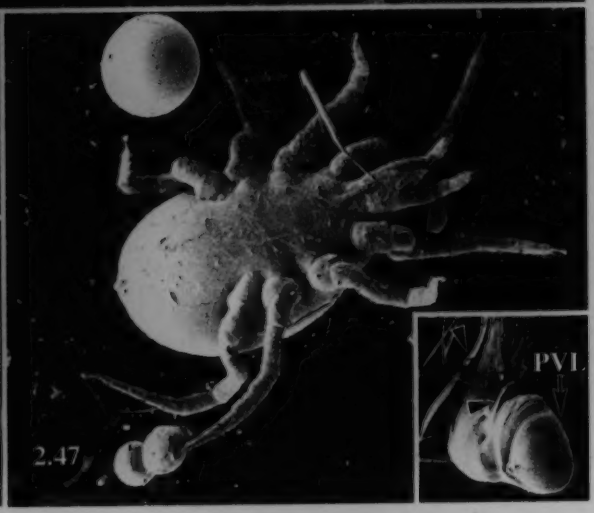


2.47

Figures 2.48 and 2.49. Pedipalps.

Figure 2.48. Setiform and peg-like mechanoreceptors on pedipalps of a *P. persimilis* adult female.

Figure 2.49. Tip of deuteronymph pedipalp showing arrangement of apical sensilla and flattened claw-like apotele.

Scale bars: Figure 2.48, 10 μm; Figure 2.49, 5 μm.

2.48

2.49

Figures 2.50-2.53. Ventral view of the gnathosoma of *P. persimilis* immatures and adults.

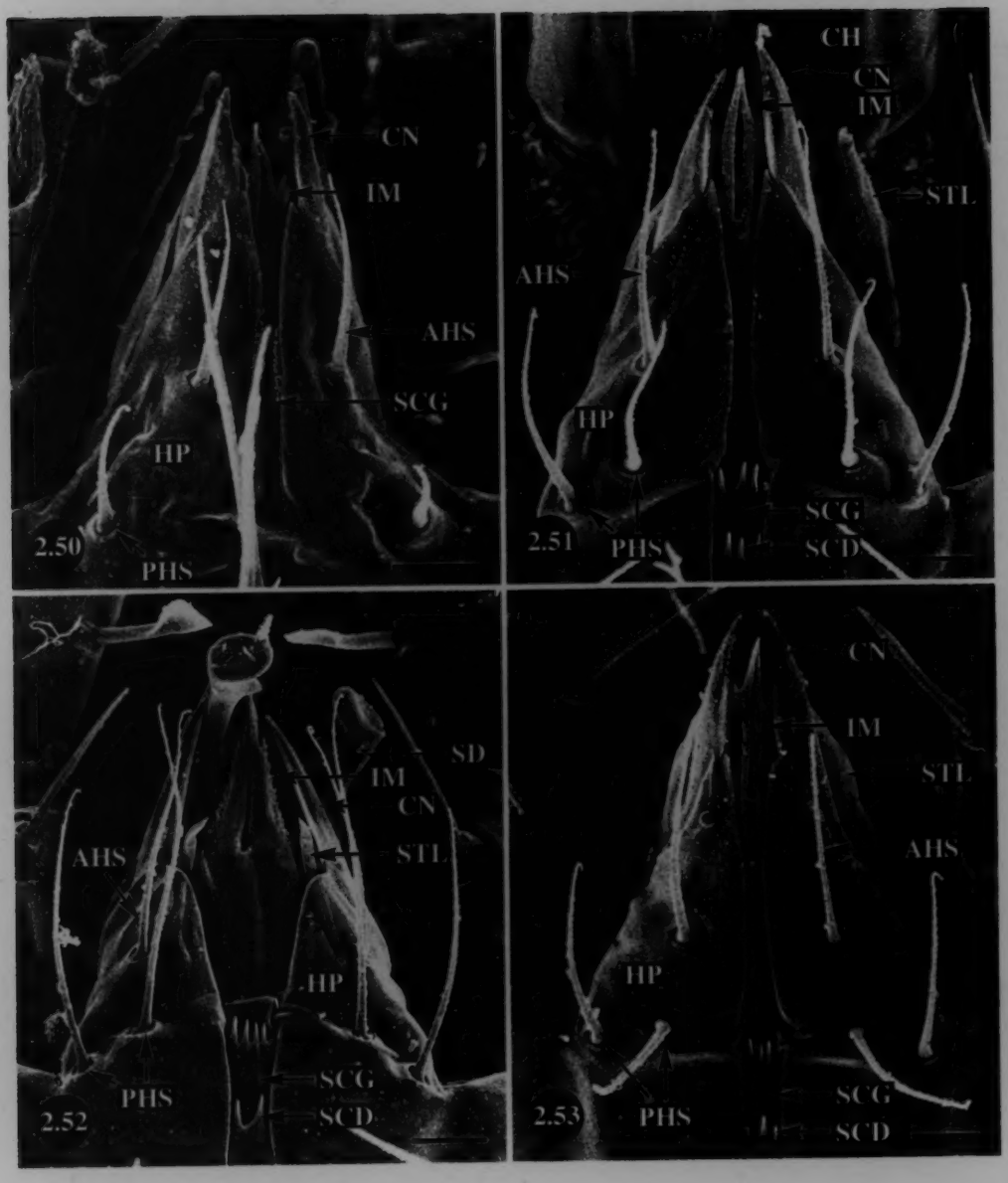
Figure 2.50. Gnathosoma of a larva. The subcapitular groove is less developed than on other stages and extends from the gnathosomal apex to the single pair of posterior hypostomatic setae.

Figure 2.51. Gnathosoma of a deuteronymph. Anteriorly directed subcapitular denticles are arranged in transverse rows within the well-defined subcapitular groove.

Figure 2.52. Gnathosoma of an adult male. The corniculi and hypostome are greatly reduced to expose the internal malae. The spermatodactyl are visible from the ventral surface.

Figure 2.53. Gnathosoma of an adult female.

Scale bars: 5 μm.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SCD

Figures 2.54-2.57. Histological preparations of the gnathosoma and anterior podosoma of *P. persimilis* adult females examined by light microscopy.

Figure 2.54. Frontal section through the gnathosoma showing orientation of labrum, corniculi and salivary styli.

Figure 2.55. Frontal section through the gnathosoma showing transverse pharyngeal dilator and constrictor muscles (arrowheads).

Figure 2.56. Frontal section through the gnathosoma showing salivary glands (dark, globular cells) on either side of the Y-shaped pharynx.

Figure 2.57. Frontal section through the gnathosoma and anterior podosomal region showing Y-shaped pharynx and oesophagus (arrowheads); the latter passes through the synganglion and divides it into the dorsal supraoesophageal ganglion and ventral suboesophageal ganglion. Microvilli are observed on the cells lining the caecal lumen.

Scale bars: Figures 2.45, 2.55 and 2.56, 10 μm; Figure 2.57, 20 μm.

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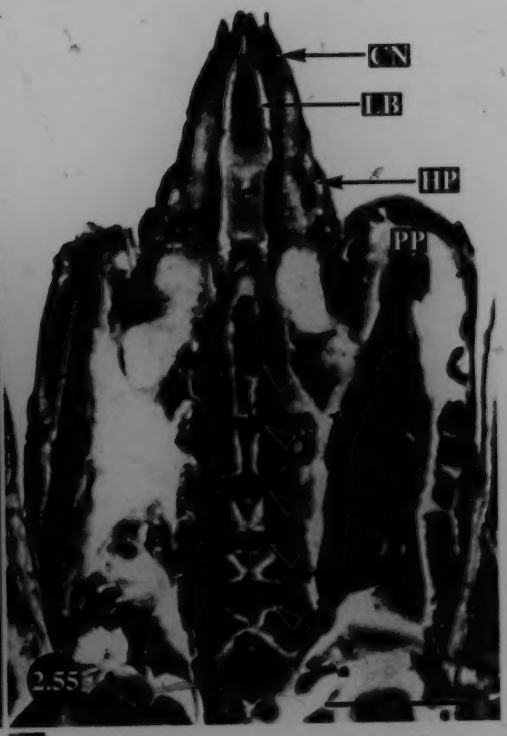
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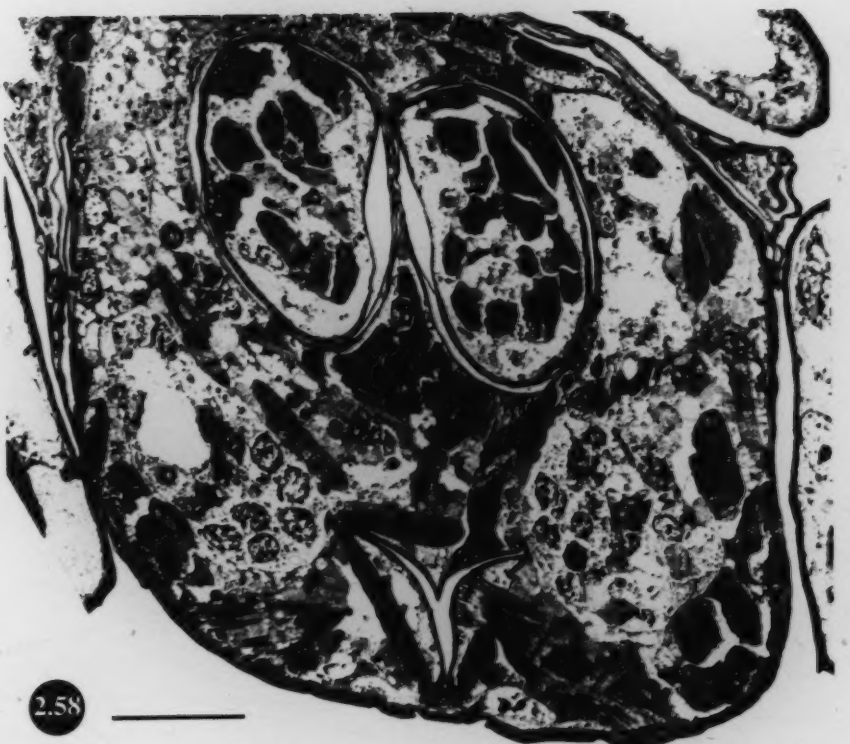
Figure 2.58. Cross section through gnathosoma of an adult female (TEM).
Figure 2.59. Diagram of cross section through gnathosoma showing gnathosomal, cheliceral and pharyngeal muscles and associated structures. Diagram is labelled according to Starovir (1973) and Akimov and Yastrebtsov (1987).

Scale bars: 10 μm.



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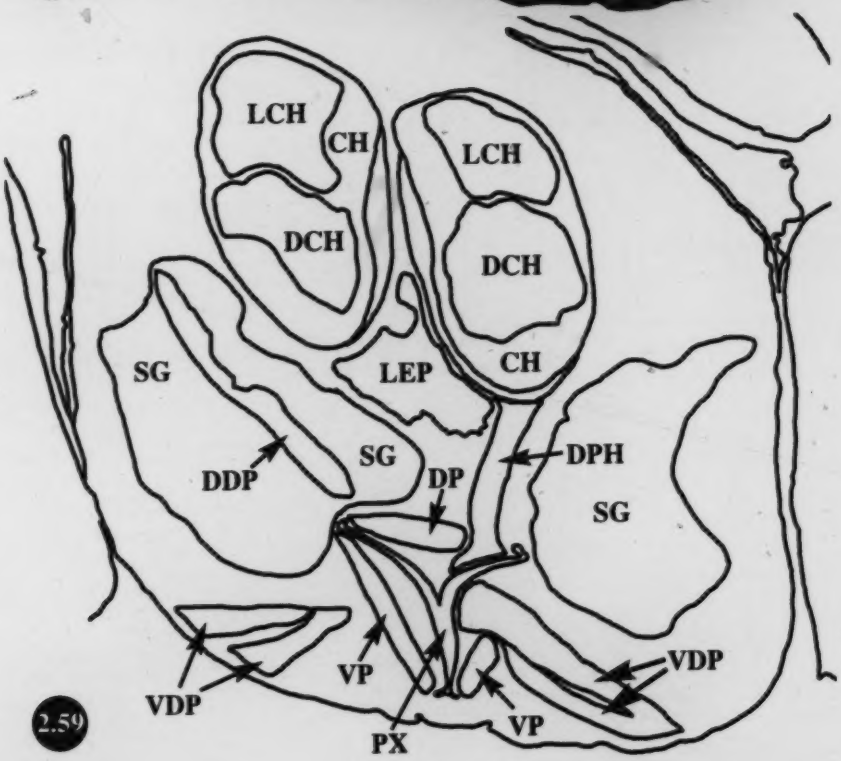


2.59

Figures 2.60-2.63. Light and transmission electron microscopic observations of the synganglion.

Figure 2.60. Frontal section through ventral gnathosoma and synganglion of an adult female (light microscopy).

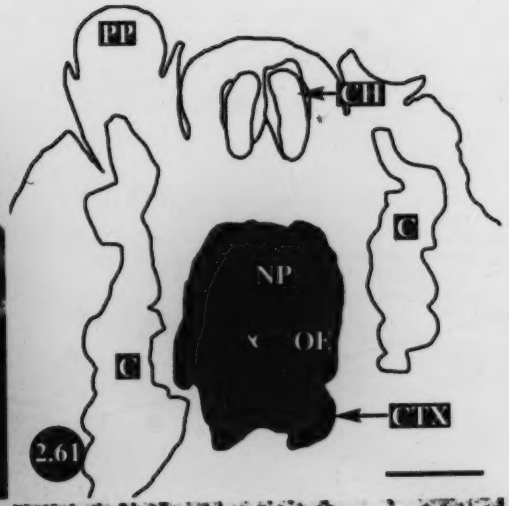
Figure 2.61. Diagram of frontal section through synganglion.

Figure 2.62. Parasagittal section through pedipalp and anterior podosoma of an adult female showing position of synganglion (light microscopy).

Figure 2.63. Synganglion: the central neuropile consists of neural fibres surrounded by the cortex (TEM).

Scale bars: Figures 2.60, 2.61 and 2.62, 20 μm; Figure 2.63, 5 μm.

2.60



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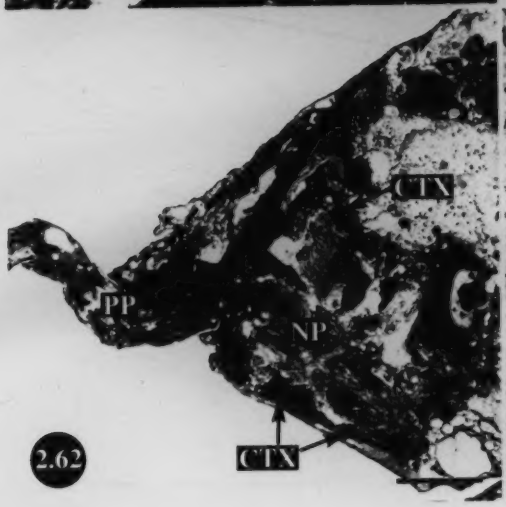

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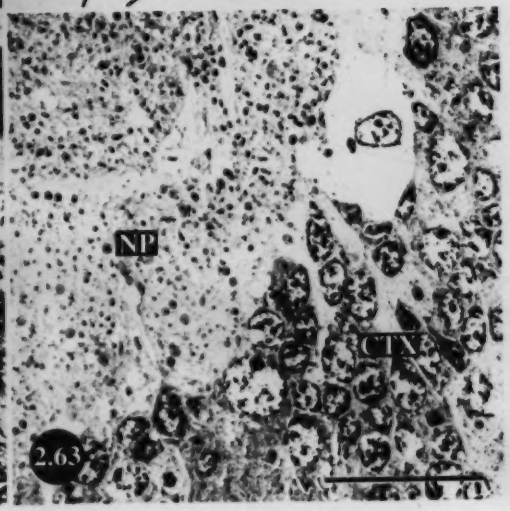


2.63

Figures 2.64-2.67. Light and transmission electron microscopic observations of the caecae and Malpighian tubules of adult female.

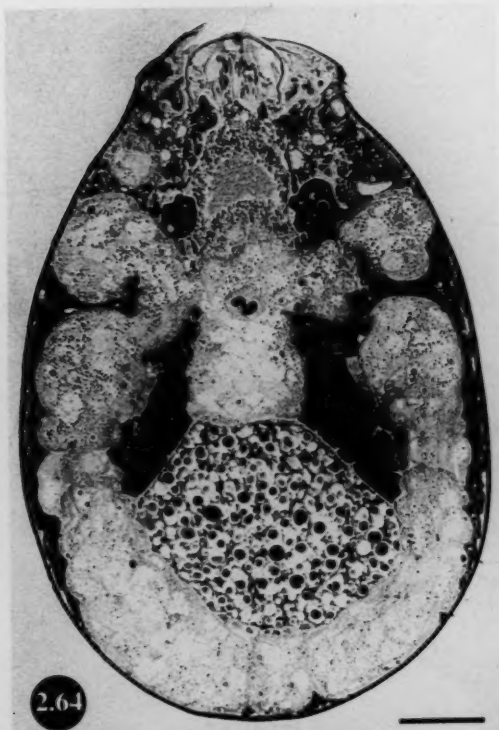
Figure 2.64. Frontal section.

Figure 2.65. Diagram of an adult female (frontal section) showing the relative position of the ventriculus, caecae, digestive cells, developing egg and synganglion.

Figure 2.66. Microvilli on cells lining the caecal lumen (TEM). Food particles and dumbbell-shaped crystals present in the caecal lumen.

Figure 2.67. Cross section of Malpighian tubule (TEM).

Scale bars: Figures 2.64 and 2.65, 20 μm; Figures 2.66 and 2.67, 5 μm.



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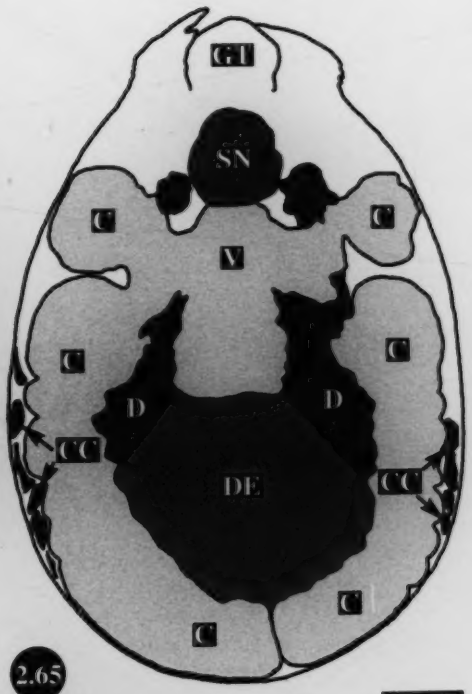

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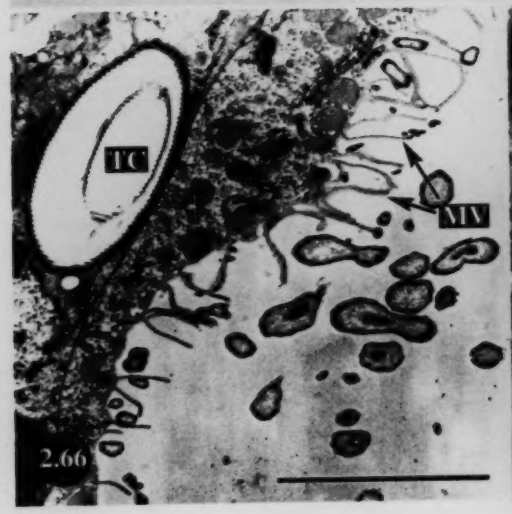

2.66

2.67

Figure 2.68. Dense mass of digestive cells on the lateral side of the ventriculus (light microscopy).

Figure 2.69. Digestive cells showing prominent, darkened nuclei and numerous vesicles (TEM).

Figure 2.70. Cross section through genital shield of an adult female showing genital orifice and genital atrium (light microscopy).

Figure 2.71. Ovarian tissue. Developing eggs (arrowheads) extend outward from the medial ovary in sac-like structures (light microscopy).

Figure 2.72. Developing egg (light microscopy).

Figure 2.73. Portion of the developing egg consisting of dark and light spherical structures (TEM).

Scale bars: Figures 2.68 and 2.73, 20 μm; Figure 2.69, 5 μm; Figures 2.70, 2.71 and 2.72, 10 μm.

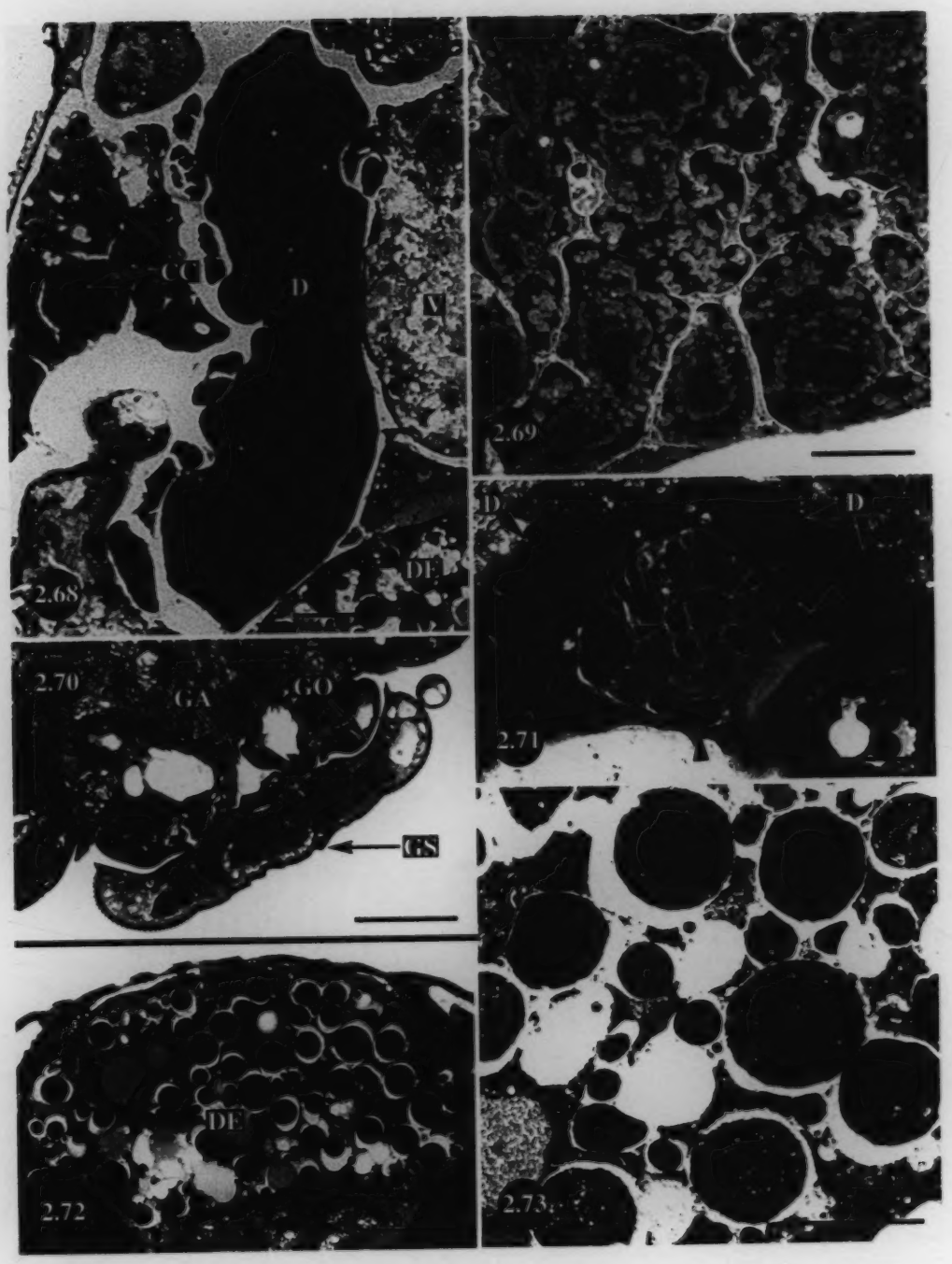
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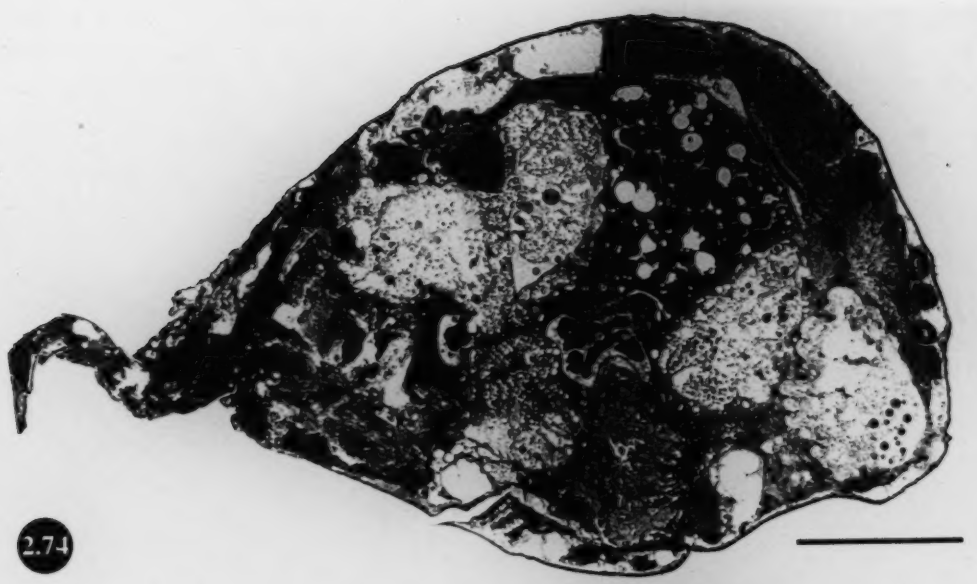
Figure 2.74. Parasagittal section of a gravid adult female.

Figure 2.75. Diagram of a gravid adult female (parasagittal) showing relative position of tissues. Identity of tissue along dorsal and lateral opisthosomal region unknown.

Figure 2.76. Cells underlying the cuticle (arrowheads); located between the cuticle and caecum (light microscopy).

Figure 2.77. Cells underlying the cuticle (TEM).

Scale bars: Figures 2.74 and 2.75, 50 μm; Figure 2.76, 10 μm; Figure 2.77, 5 μm.



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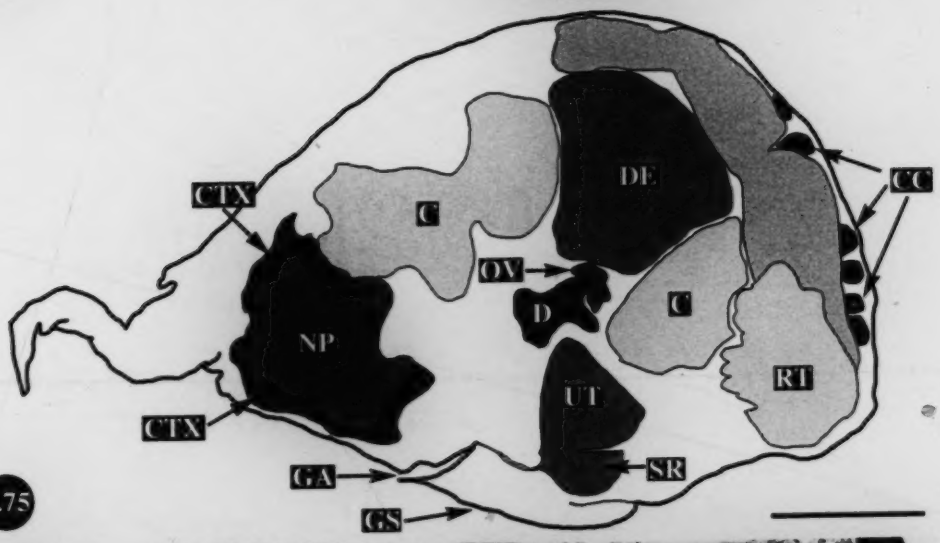

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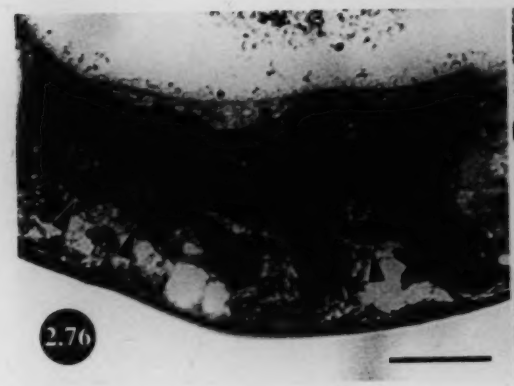

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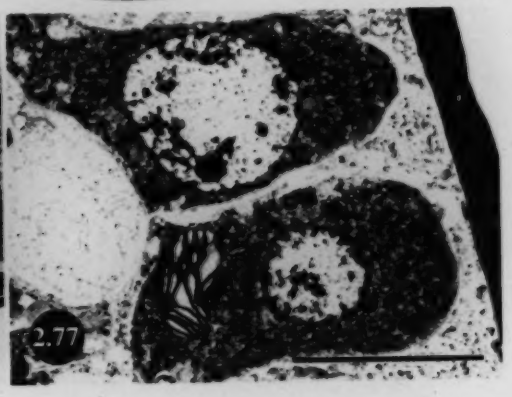
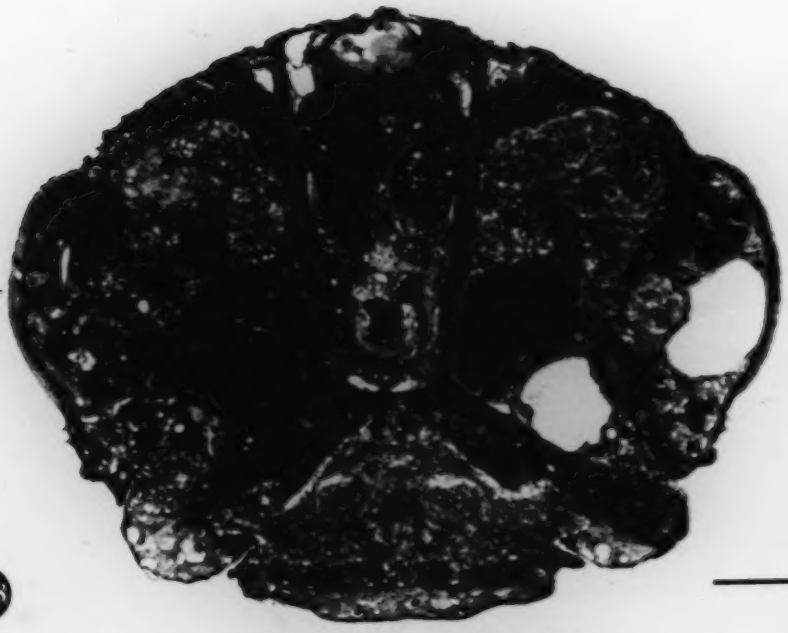


2.77

Figure 2.78. Cross section through an adult female at coxae II.
Figure 2.79. Diagram of cross section at coxae II. Identity of unlabelled tissue unknown.

Scale bars: 20 μm.



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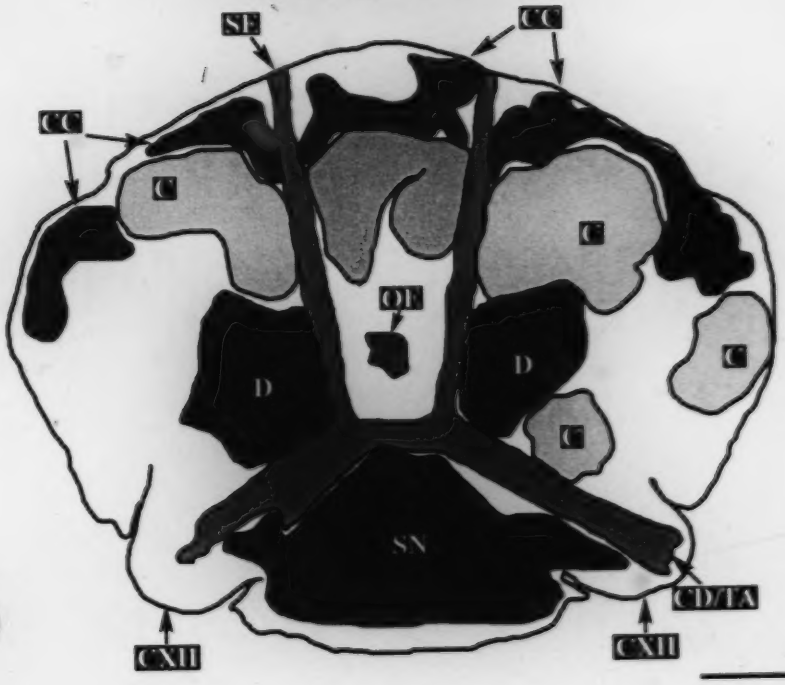
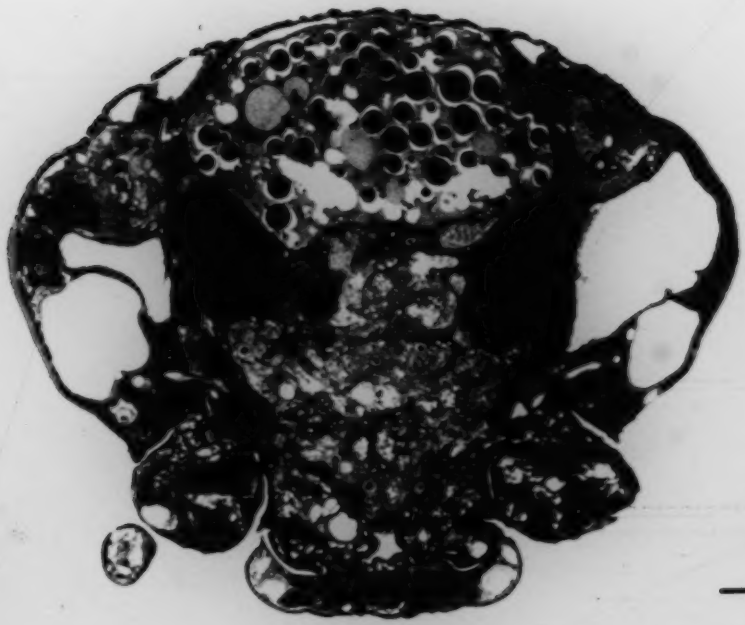


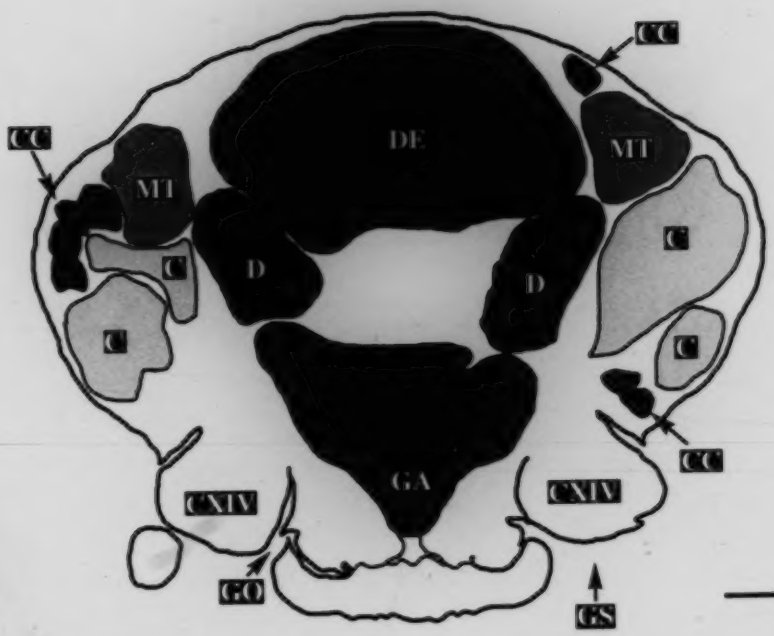
2.79

Figure 2.80. Cross section through an adult female at coxae IV.
Figure 2.81. Diagram of cross section at coxae IV. Identity of unlabelled tissue unknown.

Scale bars: 20 μm.



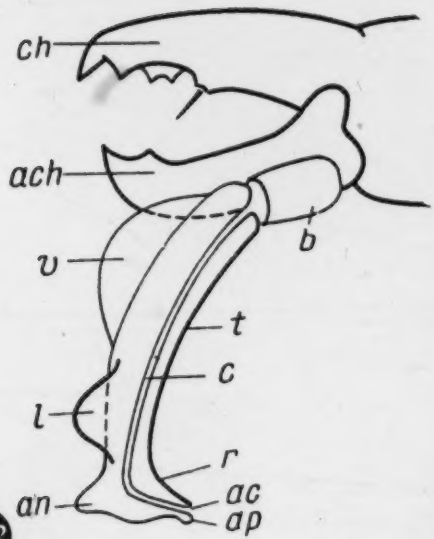
2.80



2.81

Figure 2.82. Diagram of the spermatodactyl of a male phytoseiid (Wainstein, 1973). Abbreviations: ac, aculus; ach, antichela; an, antiramus; ap, processus apicalis b, basis; c, canalis; ch, chela; l, lamellum; r, ramus; t, truncus; v, velum.

Figure 2.83. Composite diagram of the spermatodactyl of a *P. persimilis* male. Dorsal sensilla include a teardrop-shaped trichoid sensillum (arrowhead, upper right) and dorsal slit organ on each chelicera. The medial pilus dentilis and apical pit sensillum are located on the fixed digit. Two horn-like projections (arrowheads, lower middle) are positioned on each spermatodactyl arm. A single pore is located in each spermatodactyl apex.



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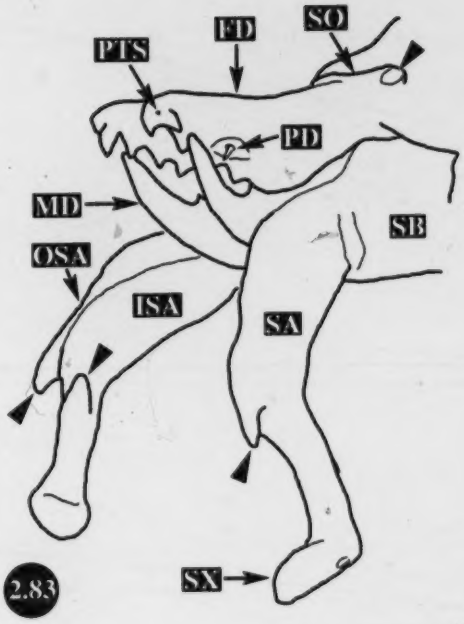

2.83

Table 2.1
Measurements of *P. persimilis* Developmental Stages

Developmental stage	Length (μm) (mean± S.E.)	Width (μm) (mean± S.E.)	n	Length (μm)†	Width (μm)†
Egg	219.23±2.73	178±1.48	10	202	177
Larva	233.06±7.86	141.02±4.77	5	221	205
Protonymph	258.44±7.64	143.04±5.98	5	269	221
Deuteronymph	293.83±6.59	161.83±7.89	3	300-348	237-269
Adult male	323.60±16.82	188.28±2.42	4	300	237
Adult female	468.04±4.09	293.44±4.56	5	363	284

† Measurements reported by Hessein (1976).

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Chapter 3
Birefringent Crystals and Abdominal Discolouration in the Predatory Mite, *Phytoseiulus persimilis* (Acari: Phytoseiidae)

Introduction

The predatory mite *Phytoseiulus persimilis* is mass-reared by commercial insectaries and shipped to growers worldwide. Growers use *P. persimilis* for biological control of spider mites in commercial greenhouses and on several field crops, including soybeans and strawberries (Schroder, 1983).

Increasing grower complaints regarding poor performance of *P. persimilis* during the 1990 growing season prompted investigation of these mites at the Alberta Research Council, Vegreville, where they were commonly found to exhibit white, abdominal discolouration. Investigation into the cause of these signs, and of the mites' poor performance, revealed several potential pathogens, including nonoccluded virus-like particles, rickettsia (genus *Wolbachia*) and microsporidia (Steiner, 1993).

Abdominal discolouration has been reported in the predatory mites *Amblyseius hibisci* (see Tanigoshi *et al.*, 1981) and *Amblyseius cucumeris*, currently known as *Neoseiulus cucumeris* (see Steiner, 1993). Opaque discolouration in Malpighian tubules and rectal plug formation were reported in *Metaseiulus occidentalis* (see Hess and Hoy, 1982). Abdominal discolouration and rectal plug formation have been ascribed to senescence (Tanigoshi, 1982). Others have speculated that these abdominal signs are diagnostic of pathogens (Schütte *et al.*, 1995). Although pathogens have been reported in *P. persimilis* and *M. occidentalis* (see Steiner, 1993; Hess and Hoy, 1982), no pathogens have ever been definitively associated with abdominal discolouration or rectal plug formation in mass-reared mites.

The aim of this study was to determine the possible correlation of potential pathogens and the occurrence of white abdominal discolouration in *P. persimilis*.

Materials and Methods

Phytoseiulus persimilis were obtained from 14 sources, both commercial and research, between 1990 and 1993: five from Europe, three from North America, three from Australia, two from Israel, and one from New Zealand.

Approximately 1000 mites from each source were imported. Asymptomatic and symptomatic *P. persimilis* were examined on arrival by light microscopy. Some mites were embedded in resin on arrival for later examination by electron microscopy; others were used to establish colonies at the Alberta Research Council, Vegreville. Colonies were maintained in cages on spider-mite (*Tetranychus urticae*)- infested bean plants, cages being placed inside greenhouses and maintained under 18L:6D; 25°C:20°C. Mites were removed from colonies periodically for examination by light and transmission electron microscopy; however, predator performance was not assessed.

Light microscopy Fresh smears of asymptomatic mites and those exhibiting abdominal discolouration were prepared in salt solution (150 mMol NaCl; 2 mMol $CaCl_2$; 3 mMol KCl). Air-dried smears were examined with a polarising filter under a light microscope. Some whole mites with abdominal discolouration were cleared in polyvinyl alcohol and examined without a polarising filter. Permanent smears of whole mites were fixed in methanol for 10 min, stained in 15% buffered Giemsa (pH 6.9) for 2 hrs, dehydrated in an ethanol series, and mounted in Permount.

Transmission electron microscopy (TEM) Whole mites were randomly selected from each source (n=20-300). Gross external signs, if present, were recorded and all selected mites were fixed in 1% paraformaldehyde and 1.5% glutaraldehyde in cacodylate buffer (pH 7.4) for 24 to 48 hr. Fixative was replaced with 0.12 M cacodylate buffer (pH 7.2) with three changes in 60 min, and tissue was postfixed for 2 hr in 1% osmium tetroxide in 0.12 M cacodylate buffer.

Fixed mites were placed in distilled water for 10 min and dehydrated in the following ethanol series: 50% (30 min), 70% (30 min), 90% (30 min), 100% (60 min), followed by propylene oxide: absolute ethanol (1:1) (30 min), propylene oxide (60 min), propylene oxide:low viscosity Spurr resin (16 hr), and low viscosity Spurr resin (24 hr). Each solution was changed three times. Individual mites were placed in Spurr resin within a flat mould and cured for a minimum of 16 hrs in a 60°C oven.

Ultrathin gold sections, approximately 100 nm thick, were cut with a diamond knife using an LKB Nova Ultramicrotome and sections were placed on Formvar-coated grids. After staining with prefiltered 4% uranyl acetate (20 min in a 60°C oven), then lead citrate (6 min at room temperature), sections were examined with an Hitachi H-600 transmission electron microscope (accelerating voltage, 75 kV) for presence/absence of dumbbells, virus-like particles, rickettsia, and microsporidia.

Scanning electron microscopy and energy-dispersive X-ray analysis Scanning electron microscopy (SEM) and energy-dispersive X-ray analysis were used to locate crystals and identify their elemental composition, respectively. Gut contents of asymptomatic mites and those exhibiting abdominal discolourations were smeared on scanning stubs covered with carbon tape. Carbon tape allowed samples to adhere to stubs without the use of fixatives that may have otherwise affected the outcome of the analysis. Smears were coated with gold.

Distinctive dumbbell shapes permitted crystals to be readily located using SEM. Only solitary crystals were examined. Once located, a high energy electron beam (accelerating voltage, 20 kV) was used to excite electrons within an area of approximately 1 μm^3 of each crystal (n=4). When electrons drop back to their steady state, they release an X-ray of energy that is equal to the distance that these have dropped. These were catalogued according to their energy, making it possible to identify elements with atomic weights greater or equal to that of carbon.

Results

General observations Asymptomatic *P. persimilis* with no visible external signs were dark orange and of uniform colour (Figure 3.1). Some immatures and adults had white, abdominal discolouration, manifested as two white bands along the dorsal side of the body in the region of the Malpighian tubules (Figure 3.2). Initial discolouration was localised to the Malpighian tubules and often extended into the forelegs. When most severe, these signs appeared as a large, centrally located white spot or as a U-shaped discolouration of the rectum in the distal opisthosoma (Figure 3.3).

Some individual mites were able to excrete the material and reverted to an asymptomatic orange colouration. In others, the material accumulated in the anus, dried, and hardened to form a rectal plug which extended from the tip of the abdomen and inhibited normal excretion. Mites with rectal plugs did not revert to a uniform, asymptomatic colouration. Affected mites appeared either dorso-ventrally flattened or remained plump and gravid in appearance. They were often lethargic. Although these signs were observed in *P. persimilis* from all sources, they were not seen in *T. urticae*, used as a food source in this study.

Light microscopy Once cleared in polyvinyl alcohol, mites exhibiting signs were found to contain densely packed, crystalline material within their Malpighian tubules (Figure 3.4), consisting of numerous, dumbbell-shaped entities. Dumbbells in Brownian movement were observed in fresh smears and these appeared birefringent when examined with polarised light. Dumbbell-shaped structures were regularly observed in stained microscopic preparations of mites from all sources, these being numerous in mites exhibiting abdominal discolouration. Asymptomatic mites had few dumbbells.

Transmission electron microscopy Dumbbells measured 2 to 4 μm long (Figure 3.5) and were found to contain prominent concentric rings when examined by TEM but with no evidence of internal cellular structure or organisation (Figure 3.6). TEM confirmed that the rectum, anal atrium, Malpighian tubules, and, occasionally, the colon of symptomatic mites were packed with dumbbell-shaped entities.

Dumbbells were also observed in mites infected with potential pathogens. Nonoccluded virus-like particles occurred exclusively in the yolk of developing eggs within gravid females (Figure 3.7). While individual mites infected with virus-like particles often had dumbbells within their Malpighian tubules, rectum, and anal atrium, virus-like particles were also observed in mites lacking dumbbells. Conversely, dumbbells were observed in mites having no visible sign of viral infection. Nonoccluded virus-like particles were observed in *P. persimilis* obtained from one European, one Australian, and three North American sources (Table 3.1).

Rickettsia were found infecting mites from seven sources: one source from Israel and two sources each from Europe, Australia, and North America (Table 3.1). DNA analysis confirmed that rickettsia found in *P. persimilis* colonies were of the genus *Wolbachia* (see Steiner, 1993). Although dumbbells were observed in mites infected with this rickettsia (Figure 3.8), not all rickettsia-infected mites contained dumbbells.

Unidentified microsporidia were found in *P. persimilis* from six sources: one source from both Israel and Europe and two sources each from North America and Australia (Table 3.1). Dumbbells were observed in both microsporidia-infected mites (Figure 3.9) and uninfected mites. Because pathogens were not always detected in mites exhibiting abdominal discolouration, the presence of birefringent crystals cannot be correlated with the occurrence of nonoccluded virus-like particles, rickettsia, or microsporidia.

Energy-dispersive X-ray analysis Energy-dispersive X-ray analysis of dumbbells detected high levels of potassium, low levels of phosphorous and sulphur and traces of chlorine (Figure 3.10). Living organisms, including bacteria and rickettsia, consist primarily of carbon, oxygen, nitrogen, and hydrogen (Campbell, 1993), therefore, if dumbbells were living matter, strong peaks representing three of these elements would be expected (hydrogen is not detected by this analysis). These, however, were not present in any significant amount.

Birefringence and internal morphology of dumbbell-shaped entities are further evidence that these are crystalline. Concentric rings observed within dumbbells are typical of crystalline inclusions.

Discussion

Abdominal discolouration in mites In previous studies, abdominal colouration in some mite species has been attributed to accumulation of either food components or nitrogenous catabolites in various locations within the digestive and excretory systems (Tanigoshi *et al.*, 1981; Steiner, 1993). As early as 1950, Hughes reported Malpighian tubules of gamasid mites to appear as white lines when viewed through the dorsal body wall. In *Cheyletus eruditus* Shrank (Trombidiformes), abdominal colouration was associated with the occurrence of

birefringent, crystalline material in excretory organs. These were thought to be normal excretory products (Hughes, 1950).

In contrast, some reports of crystalline or solid material in digestive or excretory systems of mites are associated with poor health (Hess and Hoy, 1982; Steiner, 1993). Cream to pink, rectal plugs have been observed in mass-reared mites (Tanigoshi, 1982). These were associated with motor dysfunction and reduced fecundity. Affected *A. hibisci* females exhibiting dark-red occlusions in the distal opisthosoma were dorso-ventrally flattened or concave and were lethargic and sterile (Tanigoshi *et al.*, 1981). Abdominal discolouration, similar to that in *P. persimilis*, has been associated with poor performance of the thrips predator *A. cucumeris* (see Steiner, 1993).

Birefringent crystals Crystals and associated abdominal discolouration were observed most commonly in *P. persimilis* subjected to relatively high population densities or periodic starvation while signs were less common in mites from colonies fed frequently. Rectal plugs were also common in *P. persimilis* reared under crowded conditions. These observations are consistent with those of Hughes (1950) who reported the gut contents of *Tyroglyphus farinae* L. (*Acarus siro* L.) to be birefringent when reared under crowded conditions. Hess and Hoy (1982) frequently observed rectal plug formation in dense, crowded laboratory colonies of *M. occidentalis*.

Dumbbell-shaped crystals in *P. persimilis* measured 2-4 μm long and lacked internal organelles. Schütte *et al.* (1995) reported crystalline structures of similar shape and size in *P. persimilis* from The Netherlands, these being abundant in mites that responded poorly to herbivore-induced synomones. Arutunjan (1985) described similar dumbbell-shaped entities as "bacteria" within the gut of *P. persimilis*. Šuťáková and Arutunyan (1990) speculated that these entered the gut during feeding and considered them to be symbiotic. These were similar in size, appearance, and internal structure to crystals observed within *P. persimilis*. These findings cast doubt as to whether the dumbbell-shaped entities reported by Arutunjan were bacteria.

Chemical composition The principal nitrogenous catabolite identified in

arachnids is guanine (2-amino-6-purine), with uric acid (2-6-8-oxypurine) characteristic of some species. Both guanine and uric acid are insoluble and may be stored within Malpighian tubules as crystals prior to excretion (Evans, 1992). Crystals analysed from *P. persimilis* revealed high levels of potassium, low levels of phosphorous and sulphur and traces of chlorine, elements not found in these common waste products.

Occurrence of potential pathogens Several investigators have suggested that abdominal discolouration is an indication of disease (Hess and Hoy, 1982; Schütte *et al.*, 1995), but no reports have associated specific pathogens with their formation in mass-reared mites.

Virus Birefringent crystal formation has been associated with a virus in the citrus red mite *Panonychus citri* McGregor (Smith and Cressman, 1962). Small, irregular crystals and round ones up to 50 μm in diameter were distributed throughout the body and were occasionally observed within the legs. Nonoccluded virus in *P. citri* is rod-shaped and located within midgut epithelial cells (Reed and Desjardins, 1982). Morphologically similar crystals were occasionally observed in *P. persimilis* (see Steiner, 1993); however, dumbbell-shaped crystals were not observed in midgut cells, were of a different shape, and were much smaller than the irregular and round crystals observed in *P. citri*.

Nonoccluded virus-like particles in *P. persimilis* were found exclusively in the yolk of developing eggs within gravid females but no apparent association between crystal formation and virus-like particles was established. Females with crystals were not always infected with nonoccluded virus-like particles while virus-like particles were observed in mites lacking crystals. Effects of these virus-like particles on *P. persimilis* are unknown.

Rickettsia Šuťáková and Rüttgen (1978) reported a rickettsia, *Rickettsiella phytoseiuli* Šuťáková, in *P. persimilis* obtained from Kiev. Although rickettsia of the genus *Rickettsiella* are commonly pathogenic (Tanada and Kaya, 1993), mites infected with *R. phytoseiuli* showed no developmental or morphological abnormalities.

Rickettsia of the genus *Wolbachia* reported in this study are considered

nonpathogenic; however, sublethal effects such as sterility or changes in sex ratio may occur as a result of infection. Crystals were not always observed in *P. persimilis* infected with rickettsia and only entomopathogenic rickettsia of the genus *Rickettsiella* are known to produce them (Tanada and Kaya, 1993).

Microsporidia Microsporidia are known to produce chronic, sublethal effects in their hosts that may limit their effectiveness as biological control agents (Kluge and Caldwell, 1992). Microsporidia were observed in six of the 14 colonies examined in this study. The effects of microsporidia on the performance of *P. persimilis*, however, have not been determined. *Phytoseiulus persimilis* exhibiting abdominal discolouration were not always infected with microsporidia. Conversely, microsporidia-infected *P. persimilis* showed no gross external signs or symptoms, although some gravid females were unable to oviposit (Bjørnson *et al.*, 1996).

Conclusions Although numerous birefringent crystals were observed in *P. persimilis* with abdominal discolouration, the cause of these is not yet known. Despite speculation that crystals are associated with one or several pathogens, abdominal discolouration and associated crystal formation were not correlated with the presence of microsporidia, nonoccluded virus-like particles, or rickettsia observed during this study.

Dumbbell-shaped crystals were observed in mites from all sources. Although these crystals are not common nitrogenous catabolites, they were found in low numbers in asymptomatic mites. Their excessive formation and accumulation in the rectum and Malpighian tubules of affected mites may indicate suboptimal rearing conditions such as an inadequate diet or overcrowding. Since both immatures and adults exhibited abdominal discolouration and contained birefringent crystals, these signs are not necessarily the result of senescence. Lethargy in symptomatic *P. persimilis*, along with the rectal plug formation that inhibits excretion, suggest that these signs are indicative of overall poor health.

Figure 3.1. Asymptomatic adult female *P. persimilis*.

Figure 3.2. Adult female *P. persimilis* with white discolouration within Malpighian tubules (arrow), appearing as a white stripe down the side of the body.

Figure 3.3. White, U-shaped abdominal discolouration (arrow) within distal opisthosoma.

Figure 3.4. Adult female *P. persimilis* cleared in polyvinyl alcohol. Densely packed material (arrow) is visible within the Malpighian tubules.

Figure 3.5. Scanning electron micrograph of dumbbell-shaped entities/crystals in symptomatic *P. persimilis*.

Figure 3.6. Transmission electron micrograph of dumbbell-shaped entities within the rectum. Dumbbells contain concentric rings and lack evidence of cellular organisation.

Figure 3.7. Unidentified, nonoccluded virus-like particles (arrow) in yolk of a developing egg within a gravid female. Inset: higher magnification of virus-like particles.

Figure 3.8. Rickettsia, of the genus *Wolbachia*, within unidentified tissues of *P. persimilis*.

Figure 3.9. An undescribed microsporidium in *P. persimilis*.

Scale bars: Figures 3.1, 3.2 and 3.3, 500 μm; Figure 3.4, 200 μm; Figure 3.5, 2μm; Figures 3.6, 3.7, 3.8 and 3.9, 1μm.

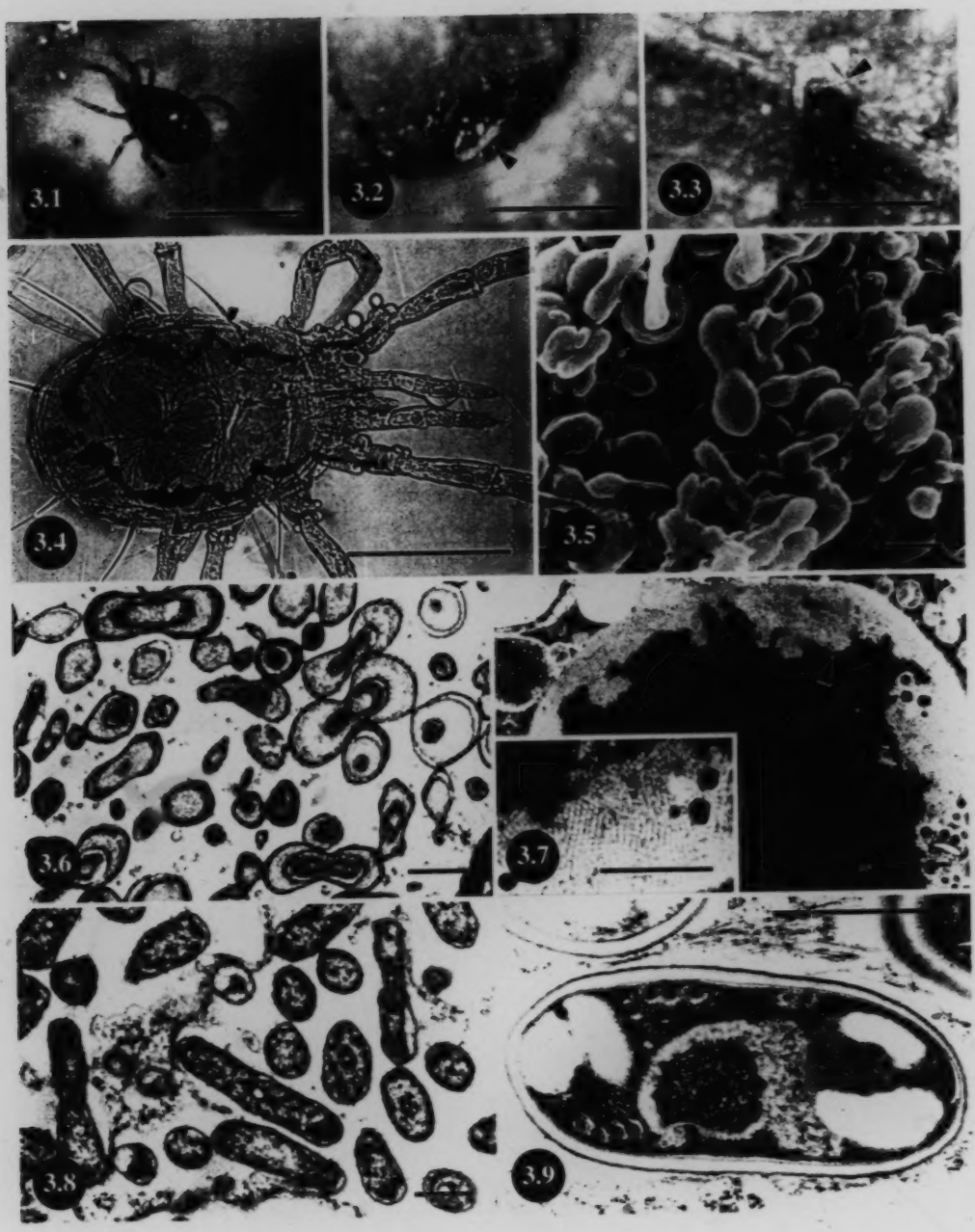
3.1
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Figure 3.10. Graph illustrating chemical composition of dumbbell-shaped entities in *P. persimilis* revealed by energy dispersive X-ray analysis (AU, gold; K, potassium; P, phosphorous; CL, chlorine; S, sulphur).

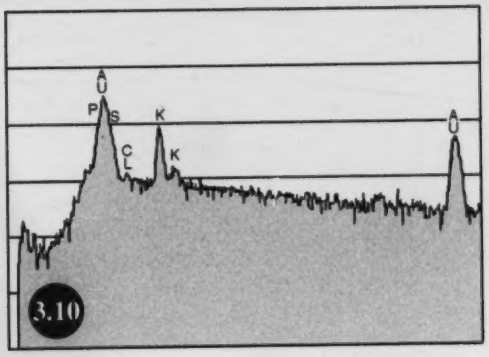
P
S
C
L
K
K
3.10

Table 3.1

Summary of Microorganisms Found Infecting *Phytoseiulus persimilis* Obtained from 14 Sources of Biological Control Agents from 1990 to 1993

No.	Location	Crystals	Virus-like particles	Rickettsia	Microsporidia	n
1.	Europe (C)	Yes (14/17)	?	Yes (5/17)	No (0/17)	17
2.	Europe (C)	Yes (1/4)	Yes (2/4)	No (0/4)	No (0/4)	4
3.	Europe (C)	Yes (4/12)	No (0/12)	No (0/12)	Yes (7/12)	12
4.	Europe (C)	Yes (26/31)	?	Yes (10/31)	No (0/31)	31
5.	Europe (R)	Yes (3/5)	?	No (0/5)	No (0/5)	5
6.	North America (C)	Yes (43/60)	Yes (7/60)	Yes (22/60)	Yes (16/60)	60
7.	North America (C)	Yes (2/10)	Yes (1/10)	Yes (1/10)	Yes (3/10)	10
8.	North America (R)	Yes (3/6)	Yes (1/6)	No (0/6)	No (0/6)	6
9.	Australia (R)	Yes (1/4)	No (0/4)	No (0/4)	No (0/4)	4
10.	Australia (C)	Yes (3/6)	Yes (1/6)	Yes (1/6)	Yes[a] (0/6)	6
11.	Australia (C)	Yes (1/5)	?	Yes (1/5)	Yes[a] (0/5)	5
12.	Israel (C)	Yes (4/7)	No (0/7)	Yes (2/7)	Yes (5/7)	7
13.	Israel (C)	Yes (2/7)	No (0/7)	No (0/7)	No (0/7)	7
14.	New Zealand (R)	Yes (1/2)	No (0/2)	No (0/2)	No (0/2)	2
Percentage of Total		61.4	6.8	23.9	17.6	

Note. n = 2-60; microorganisms observed with transmission electron microscopy, unless stated otherwise. ?, data inconclusive; C, commercial insectary; R, research colony.

[a] observed with light microscopy only.

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Chapter 4
Ultrastructure and Pathology of *Microsporidium phytoseiuli* n. sp. Infecting the Predatory Mite, *Phytoseiulus persimilis* (Acari: Phytoseiidae)

Introduction

The predatory mite *Phytoseiulus persimilis* Athias-Henriot is mass-reared by commercial insectaries and shipped to growers worldwide. Growers use this mite for biological control of two-spotted spider mite, *Tetranychus urticae*, in commercial greenhouses and on field crops. *Phytoseiulus persimilis* is an effective predator and its ability to control pest mite populations in greenhouses is well documented (Scopes, 1985). However, recent grower complaints regarding poor performance of this predator in Alberta greenhouses led to the discovery of several potential pathogens associated with it, including microsporidia (Steiner, 1993a).

Microsporidia have been reported from both aquatic and terrestrial mites. Other phytoseiid hosts include *Neoseiulus cucumeris* (formerly *Amblyseius*) and *Amblyseius barkeri*, predators used for biological control of western flower thrips, *Frankliniella occidentalis* Pergande, and onion thrips, *Thrips tabaci* Lindeman, in commercial greenhouses (Beerling *et al.*, 1993; Steiner, 1993a). Microsporidia reduced the productivity of mass-rearings of these mites (Beerling and van der Geest, 1991) and were implicated in reducing fecundity and longevity in *P. persimilis* (see Steiner, 1993b).

Based on spore morphology alone, three distinct microsporidia were observed in *P. persimilis*, obtained from three different, commercial insectaries. The ultrastructure and pathology of one of these microsporidia are described from light and transmission electron microscopy data.

Materials and Methods

Mite rearing *Phytoseiulus persimilis* used for this study were obtained from a supplier of biological control agents in Europe in December 1993 and reared at the Alberta Research Council (Vegreville, AB). Colonies of mites were maintained in cages on bean plants (*Phaseolus vulgare*) infested with two-spotted spider mites, *T. urticae*. Cages were placed within greenhouses under controlled environmental conditions (18L:6D; 25°C:20°C).

Light microscopy Fresh smears of whole *P. persimilis* and *T. urticae* were prepared in salt solution (150 mM NaCl; 2 mM $CaCl_2$; 3 mM KCl) and measurements of fresh spores were obtained by the agar cushion method of Hostounský and Žižka (1979). Permanent smears of whole mites were fixed in methanol for 10 min, stained in 15% Giemsa buffer (pH 6.9) for 2 hr, dehydrated in an ethanol series, and mounted in Permount.

Transmission electron microscopy Adult female *P. persimilis* were placed in fixative consisting of 1% paraformaldehyde and 1.5% glutaraldehyde in cacodylate buffer (pH 7.4) for 24 to 48 hr. Fixative was replaced with 0.12 M cacodylate buffer (pH 7.2) with three changes in 60 min. Tissue was postfixed for 2 hr in 1% osmium tetroxide in 0.12 M cacodylate buffer.

Following fixation, mites were placed in distilled water for 10 min and dehydrated in the following ethanol series: 50% (30 min), 70% (30 min), 90% (30 min), 100% (60 min), followed by propylene oxide:absolute ethanol (1:1) (30 min), propylene oxide (60 min), propylene oxide:low-viscosity Spurr resin (16 hr), and low-viscosity Spurr resin (24 hr). Each solution was changed three times. An individual mite was placed in Spurr resin within a flat mould and cured for a minimum of 16 hr in a 60°C oven.

Ultrathin gold sections, approximately 100 nm thick, were cut with a diamond knife using an LKB Nova Ultramicrotome. Sections were placed on Formvar-coated grids and stained with prefiltered, 4% uranyl acetate for 20 min in a 60°C oven and then with lead citrate for 6 min at room temperature. Sections were examined with an Hitachi H-600 transmission electron microscope (accelerating voltage, 75 kV).

Results

Presporal stages

Distinct developmental stages of the microsporidium were not observed in light microscopic examination of stained preparations. All stages observed with the electron microscope were uninucleate. The earliest stage observed was the schizont. Rounded-to-ovoid schizonts measured 1.9-2.9 μm (Figure 4.1). The nucleus (N) occupied a large portion of schizonts and cisternae of rough

endoplasmic reticulum (ER) and Golgi apparatus (G) were commonly observed within them. Cytoplasm contained densely packed ribosomes. Several schizonts were commonly observed within a single host cell (Figure 4.2). Those developing within host cell nuclei were typically in chromatin-rich regions adjacent to the nuclear membrane.

Individual cells suspected of being sporonts contained rough endoplasmic reticulum, a single Golgi apparatus, and abundant, loosely packed, ribosomes. These cells, although presumed to be sporonts, could not be confidently distinguished from schizonts. Plasmodia were not observed, suggesting that both schizogony and sporogony may be the process of binary division.

Sporoblasts were more elongate than earlier stages and measured up to 5.6 μm long, making them comparable in size to mature spores. Each was surrounded by a double-layered, electron-dense plasma membrane. Sporoblasts were often observed with a caudal prolongation (Figure 4.3). These appeared to lack a double-layered membrane, likely an artifact resulting from incomplete fixation.

The mature spore

Mature spores were broad- to elongate-ovoid (Figures 4.4 and 4.5) and measured 4.33±0.35 x 1.27±0.15 μm in electron micrographs (n=13). Fresh spores measured 5.88±0.34 x 2.22±0.19 μm (n=30), while those fixed in methanol and Giemsa stained measured 5.37±0.46 x 2.22±0.17 μm (n=30).

An isofilar polar filament (PF) coiled 12 to 15 times in the posterior two-thirds of the spore (Figures 4.4 and 4.5). The base of its anchoring disc (AB) measured 150 nm, with long, slender arms (AA) each measuring approximately 250 nm in length (Figure 4.6). A lamellar polaroplast (P) occupied approximately one-third of the anterior region of the mature spore. Lamellae in the posterior portion of the polaroplast were more loosely packed than those in the anterior part. Large cavities in the polaroplast are artifacts, probably resulting from incomplete fixation. All spores had a relatively large posterior vacuole (PV) occupying about one-fourth of the spore (Figures 4.4 and 4.5).

Spore walls were relatively uniform in thickness but thinner at the anterior end. Measurements from micrographs showed them to be 89-101 nm thick and to

consist of three layers: an innermost plasma membrane (PM), a translucent endospore (EN) layer measuring 54-65 nm, and an electron-dense exospore (E) measuring 30-36 nm (Figure 4.7).

Spores were occasionally observed within envelopes of unknown origin. These are referred to as interfacial envelopes after the definition of Sprague *et al.* (1992). Fresh smear preparations examined by light microscopy revealed few interfacial envelopes, some of which persisted as sporophorous vesicles. These often contained four, eight, to more than 16 spores (Figure 4.8).

Both individual spores and spores within interfacial envelopes were observed by transmission electron microscopy. Individual spores, apparently in direct contact with host cytoplasm, were observed throughout all infected mite tissues, including supra- and suboesophageal ganglia, muscle tissue, parenchyma, digestive cells, and cells lining the caecal lumen. Interfacial envelopes, observed only in parenchyma cells underlying the cuticle (Figures 4.9 and 4.10), contained up to eight spores.

Pathology

Microsporidian spores were not observed in prey mites (n=500) taken directly from rearing colonies nor in partially consumed mites (n=200) exposed to infected *P. persimilis*. Microsporidia-infected *P. persimilis* showed no gross external signs or symptoms, although some gravid females were unable to oviposit. Infected mites often appeared lethargic, and, occasionally, moribund mites rapidly retracted their legs and twitched when disturbed.

Schizonts occurred in digestive cells, in the cytoplasm of cells lining the caecal wall or in its underlying muscle tissue. Schizonts were commonly observed within nuclei of digestive cells in the chromatin-rich region of a nucleus adjacent to the nuclear membrane (Figure 4.11), but occasionally outside nuclei of these cells. Sporont-like cells and sporoblasts occurred within nuclei of digestive cells, and mature spores in both cytoplasm and nuclei. Evacuated spores, remnants of spores after sporoplasm discharge, were occasionally observed in digestive cell nuclei. Sporont-like cells, sporoblasts, and mature spores were not restricted to digestive cells as these were commonly observed in cells lining the caecae. Both

mature spores and sporoblasts were observed simultaneously within individual cells.

Infected cells sloughed into the caecal lumen contained developmental stages and mature spores, in addition to food particles and bacteria (Figure 4.12). Bacteria were not observed in all specimens examined and are therefore considered secondary pathogens.

Numerous mature spores were observed within cells of the caecal wall. Once infected with microsporidia, these cells hypertrophy and break away from the caecae, damaging the integrity of the caecal wall (Figure 4.13). Severe infection caused extensive damage to the caecal wall, including loss of all microvilli and occasional secondary infection by opportunistic bacteria (Figure 4.14). Spores were also observed within muscle fibres (Figure 4.15), within the cortex of the sub- and supraoesophageal ganglia (Figure 4.16), and within developing eggs of gravid females in direct contact with yolk.

Discussion

The microsporidium in *P. persimilis* had unpaired nuclei for all stages of development observed. Spores were broad- to elongate-ovoid, with single ones apparently in direct contact with host cytoplasm. Interfacial envelopes contained four, eight, to more than 16 spores. Variability in spore shape, infrequent occurrence of interfacial envelopes (which may be an artifact due to incomplete fixation) and the presence of schizonts in both nuclei and cytoplasm of host cells raise the possibility that more than one species of microsporidia was present.

Host cell nuclei are rarely invaded by microsporidia (Canning, 1990), although several exceptions have been observed. *Nosema apis* (in the honeybee, *Apis mellifera*), *Nosema fumiferanae* Thom. (in spruce budworm, *Choristoneura fumiferana*) and *Nosema bombycis* Naegeli (in the silkworm, *Bombyx mori* L.) have been observed within cell nuclei of their respective hosts (Tanada and Kaya, 1993). *Nosema* sp. was detected in nuclei and cytoplasm of midgut muscle cells in the parasitic wasp *Pediobius foveolatus* Crawford (Chapman and Hooker, 1992), and *Steinhausia mytilovum* Field, a microsporidium infecting the nurse cells of ovaries and oenocytes of the mussel, *Mytilus edulis* L., occasionally infects host

nuclei (Sprague *et al.*, 1992). *Enterocytozoon salmonis* Chilmonczyk, Cox and Hedrick and *Microsporidium rhabdophilia* Modin develop exclusively within cell nuclei of salmonid fish (Chilmonczyk *et al.*, 1991).

Although infected nuclei often hypertrophy, protein synthesis necessary for cells to grow and accommodate maturing parasites continues (Canning, 1990). In *P. persimilis*, infection of digestive cell nuclei was clearly observed but their premature death may be insignificant, as undifferentiated cells in the ventriculus are continuously maturing and replacing them. In starved, uninfected mites, few mature digestive cells are present in the gut (Chant, 1985). Shortly after ingestion of food, undifferentiated cells mature and become more vacuolated. These absorb food particles and are eventually sloughed into the caecal atrium. Degeneration of mature cells occurs rapidly and sloughed ones are replaced continually during digestion (Akimov and Starovir, 1974).

Evacuated spores observed in the nuclei of digestive cells are evidence for autoinfection. Sloughing of infected cells into the caecal lumen may disseminate microsporidia throughout the digestive system. The presence of schizonts in the caecal wall and in its underlying muscle tissue supports the assumption that autoinfection of the caecae does occur. Extensive damage and numerous mature spores within the caecal wall further indicate autoinfection. Secondary infection by opportunistic bacteria is occasionally observed when wall damage is extensive.

Destruction and sloughing of cells of the caecal wall may lead to reinfection of the same host and infection of new ones. Once in the caecal atrium, spores may be excreted with metabolic waste onto foliage and provide inoculum necessary for horizontal transmission. Mature spores have also been observed in developing eggs within gravid females and these may provide a means for vertical transmission.

Microsporidia previously reported to infect aquatic and terrestrial mites are listed in Table 4.1. The first reported occurrence of microsporidiosis in mites was by Weiser (1956) of *Nosema steinhausi* Weiser in the phytophagous mite *Tyrophagus noxius* Zakhvatkin. A second species, *N. sperchoni* Lipa, was identified from a single specimen of the water mite, *Sperchon* sp. (Lipa, 1962). A highly

pathogenic microsporidium, *Gurleya sokolovi* Issi and Lipa was found in haemocytes, nerve cells, and adipose tissue of the water mite, *Limnochares aquatica* L. (Issi and Lipa, 1968), and a second microsporidium, *Napamichum aequifilum* Larsson, was reported to infect the same mite (Larsson, 1990). Hazard and Oldacre (1976) described *Cryptosporina brachyfila* Hazard and Oldacre in adipose tissue of *Piona* sp., a water mite.

The first description of microsporidiosis in oribatid mites was recorded by Purrini and Bäumler (1976) with *Nosema ptyctimae* Purrini and Bäumler in the fat body and nephrocytes of *Rhysotritia ardua* C.L. Koch. *Nosema euzeti* Lipa was reported to infect both larvae and adults of *Euzetes seminulum* O.F. Müller (Lipa, 1982).

In 1981, Purrini and Weiser found 12 species of moss mites to be infected with eight distinct microsporidia of three genera. Four belonged to the genus *Pleistophora*, three to *Nosema* and a single species to *Thelohania*. Microsporidia in the Family Pleistophoridae have been reported to infect *N. cucumeris* (formerly *Amblyseius*) and *A. barkeri*, two phytoseiid mites used for biological control of western flower thrips, *Frankliniella occidentalis*, and onion thrips, *Thrips tabaci*, in commercial greenhouses. In this instance, the phytophagous mites *Acarus siro* and *Tyrophagus putrescentiae* Schrank, which serve as food for *A. cucumeris* and *A. barkeri* in mass rearing, were infected with the same microsporidian species (Beerling and van der Geest, 1991; Beerling *et al.*, 1993). Microsporidia were not detected in prey mites used in this study.

Only *Pleistophora platynothri* Purrini and Weiser, in the oribatid mite *Platynothrus peltifer* C.L. Koch (Purrini and Weiser, 1981), has uninucleate spores with measurements similar to those found in *P. persimilis* (Table 4.1). The microsporidium in *P. persimilis*, therefore, represents a previously undescribed species. Incomplete information on the life cycle and development of this microsporidium makes it difficult to ascribe it to an existing genus. For this reason this microsporidium is assigned to the collective group *Microsporidium* and is given the specific name *phytoseiuli*.

Taxonomic summary

Type host. *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae).

Transmission.[1] Unknown. Evidence of autoinfection in digestive cell nuclei and caecae. Mature spores observed in developing eggs within gravid females may provide a means for vertical transmission.

Site of infection. Nuclei of digestive cells, caecal wall, muscle tissue, cortical regions of sub- and supraoesophageal ganglia, parenchyma cells underlying the cuticle, and within developing eggs.

Interface. Schizonts observed almost exclusively in digestive cell nuclei: sporont-like cells, sporoblasts, and spores in direct contact with host cytoplasm. Groups of four, eight, to more than 16 spores occasionally observed within envelopes of unknown origin.

Other parasite-host cell relations. Parasites occur in both host cell nucleus and cytoplasm. Hypertrophy of infected caecal wall cells.

Development. All stages observed had unpaired nuclei. Schizogony and sporogony not recognized.

Spore. Broad- to elongate-ovoid. Fresh spores are 5.88±0.34 x 2.22±0.19 μm, Giemsa-stained spores are 5.37±0.46 x 2.22±0.17 μm, and spores from transmission electron micrographs are 4.33±0.35 x 1.27±0.15 μm. Spores are uninucleate. An isofilar polar filament coils 12 to 15 times. Polaroplast lamellar. Spore walls relatively uniform in thickness (89-101 nm) but thinner at the anterior end. Exospore moderately developed (30-36 nm) and endospore relatively thick (54-65 nm). Spores occasionally observed within envelopes of unknown origin. Groups of four, eight to more than 16 spores.

Type locality. Biological control agents obtained from a commercial insectary in Europe.

Deposition of type specimens.[2] Retained by the author (S.B.).

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[1] Vertical and horizontal transmission studies were completed following the publication of this chapter. Results of these experiments are reported in Chapter 7.

[2] Type specimen to be submitted to the Smithsonian Institute.

Figures 4.1-4.5. Developmental stages of the microsporidium in *P. persimilis*.

Figure 4.1. Schizont.
Figure 4.2. Schizont in an unidentified host cell, showing numerous host mitochondria.
Figure 4.3. Sporoblast, showing early development of the polar filament.
Figure 4.4. Mature spore.
Figure 4.5. Mature spore showing variability in shape and size.

Scale bars: 1 μm.

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M
4.2
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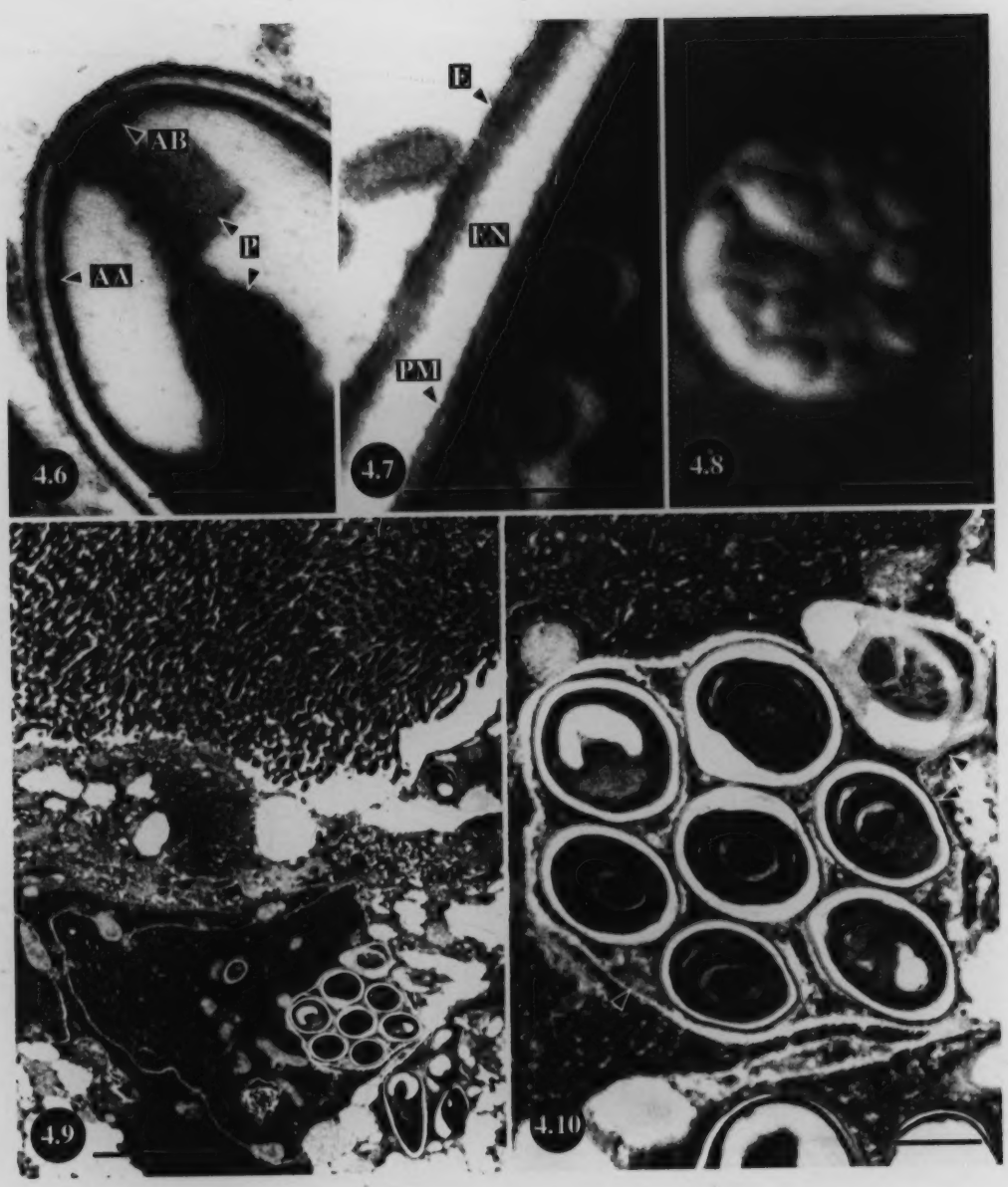
4.3
4.4
A
PF
P
N
R
PV
4.5

Figures 4.6-4.10. Characteristics of mature spores.

Figure 4.6. Polar filament/anchoring disc detail.
Figure 4.7. Spore wall detail.
Figure 4.8. Sporophorous vesicle containing many spores (fresh smear preparation, light microscopy).
Figure 4.9. Spores within an interfacial envelope within a parenchyma cell, underlying host cuticle.
Figure 4.10. Interfacial envelope (arrowheads) enveloping eight spores.

Scale bars: Figure 4.6, 0.5 μm; Figure 4.7, 0.25 μm; Figures 4.8 and 4.9, 5 μm; Figure 4.10, 1 μm.


AB
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4.8
4.9
4.10

Figures 4.11-4.16. Pathology caused by the microsporidium in *P. persimilis*.

Figure 4.11. Development of schizonts (arrowheads) in nucleus of digestive cell.
Figure 4.12. Host cell containing microsporidian spores and bacteria, sloughed off into caecal atrium.
Figure 4.13. Microsporidian spores within cells of caecal wall, causing cell hypertrophy and sloughing of cells into caecal atrium. Schizonts (arrowheads) observed in cells of caecal wall and muscle fibres underlying the caecal wall.
Figure 4.14. Secondary infection of bacteria in caecum and destruction of caecal wall.
Figure 4.15. Mature spores within muscle fibre. Schizonts in digestive cell nucleus (arrowheads).
Figure 4.16. Microsporidian spores in cortex of suboesophageal ganglion.

Scale bars: 5 μm.

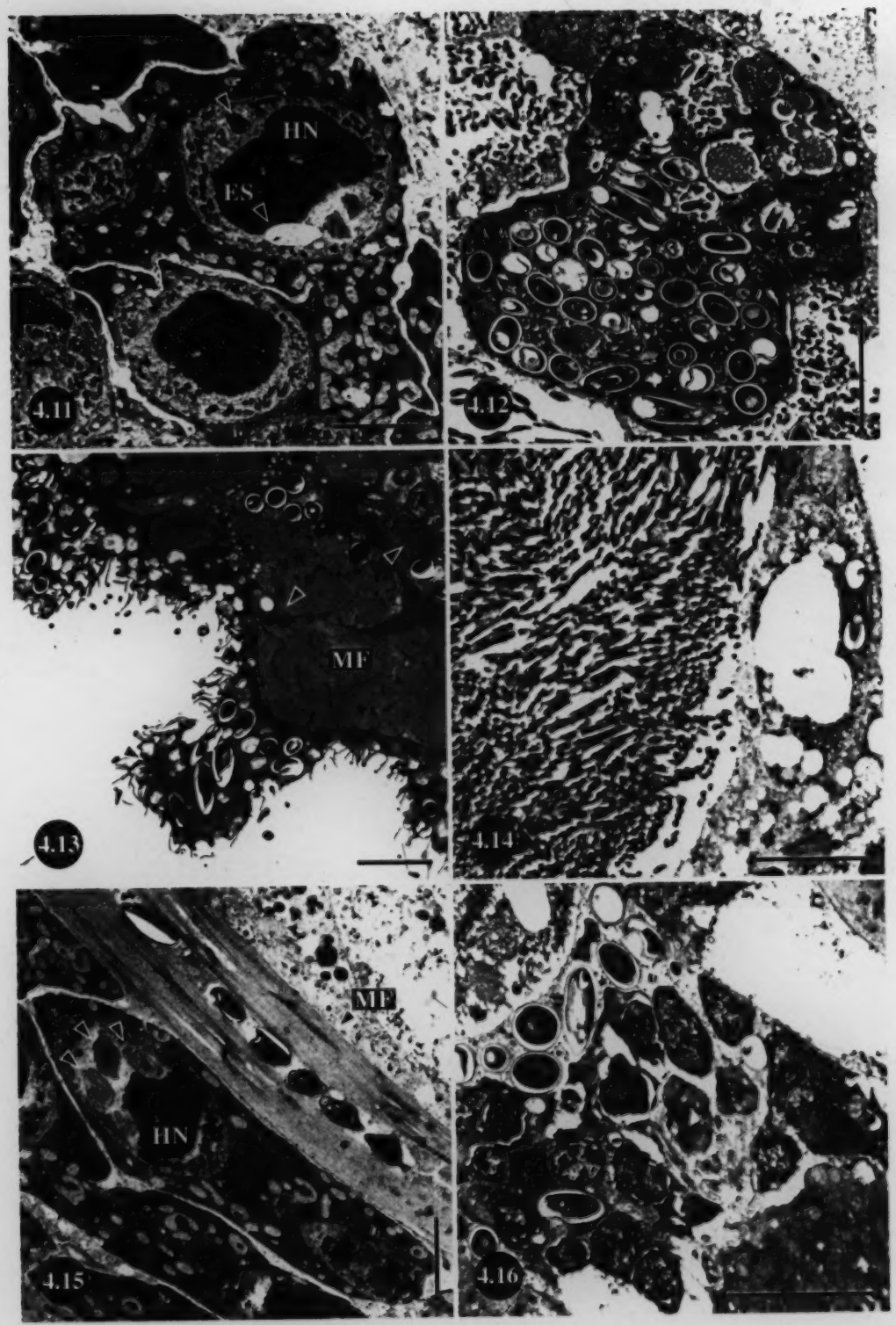
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Table 4.1
Microsporidia Reported in Aquatic and Terrestrial Mites

Author	Host	Microsporidium	Spore dimensions (μm)
Weiser (1956)	*Tyrophagus noxius* Zakhvatkin	*Nosema steinhausi*	2.2-3 x 1-1.5
Davies (1960)	*Sperchon* nr. *jasperensis* Marshall	*Nosema* sp.	(?)
Lipa (1962)	*Sperchon* sp.	*Nosema sperchoni*	4.5-5.5 x 3.0-3.5
Issi and Lipa (1968)	*Limnochares aquatica* L.	*Gurleya sokolovi*	6.9-7.5 x 3.5-4.4 (fresh) 4.5-6.6 x 1.9 (fixed/stained)
Hazard and Oldacre (1976)	*Piona* sp.	*Cryptosporina brachyfila*	1.80-1.91 x 0.53-0.80
Purrini and Bäumler (1976)	*Rhysotritia ardua* C.L. Koch	*Nosema ptyctimae*	4.4-4.8 x 1.6-1.8
Purrini and Weiser (1981)	Oribatid mites	*Pleistophora oribatei*	2.0-2.5 x 1.0-1.5
		Pleistophora cephei	3.0-3.5 x 2.0-2.5
		Pleistophora platynothri	4.5-5.0 x 2.5
		Pleistophora dindali	2.0.-2.5 x 1.8-2.0
		Thelohania microtritiae	1.8-2.0 x 1.5
		Nosema acari	4.0-5.5 x 2.2-2.5
		Nosema steganacari	3.5-4.0 x 2.0-2.5
		Nosema führeri	2.5-3.0 x 2.0
Lipa (1982)	*Euzetes seminulum* O.F.Müller	*Nosema euzeti*	3.1-4.3 x 1.9-2.5 (fixed/stained) 3.2-4.7 x 1.9-2.6 (fresh)
Larsson (1990)	*Limnochares aquatica*	*Napamichum aequifilum*	5.4-5.8 x 3.0 (unfixed) 4.0-5.0 x 2.8-3.0 (fixed/stained)
Beerling *et al.* (1993)	*Amblyseius cucumeris* Oudemans *Amblyseius barkeri* Hughes *Acarus siro* L. *Tyrophagus putrescentiae* Shrank	Fam: Pleistophoridae	1.8 x 0.9

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Chapter 5
A Descriptive Comparison of Microsporidia Found Infecting *Phytoseiulus persimilis* from Three Commercial Sources

Introduction

Recent reports of pathogens within populations of mass-reared arthropods have raised questions regarding the quality of biological control agents used for commercial pest control. Microsporidia, spore-forming protozoan pathogens, are the most frequently reported pathogens in mass-reared hymenopterous parasitoids and acarine predators. Because spore infectivity and persistence for most species of microsporidia have not been investigated (Maddox, 1973), microsporidia are of particular concern in mass-rearings within commercial insectaries and laboratories.

Microsporidia were observed in *Phytoseiulus persimilis* obtained from commercial sources during routine examination of individuals for pathogens. Analysis of spore morphology indicated that three species of microsporidia had infected *P. persimilis* obtained from three different commercial sources. One microsporidium was found within predators obtained from a North American insectary, while a second species was observed within predators obtained from a commercial source in Israel. The aim of this study was to describe differences in the life stages, spore morphology, and pathology of these two microsporidian species. The development and pathology of these two species are then compared to those of *Microsporidium phytoseiuli* Bjørnson, Steiner and Keddie found infecting a population of *P. persimilis* obtained from a commercial European source. The ultrastructure and pathology of *M. phytoseiuli* are described in Chapter 4.

Materials and Methods

Phytoseiulus persimilis were obtained from commercial suppliers in North America in November 1990, and from Israel in July 1991. Twenty-five specimens from each source were examined by light microscopy and another 25 were prepared for transmission electron microscopy (TEM). Due to specimen loss during infiltration procedures, only four specimens from North America and 19 from Israel were embedded. Of these, two North American specimens were suitable for examination by TEM, while five from Israel were examined. Parasite

transmission and effects on host performance were not determined due to unavailability of live specimens from these sources.

Transmission electron microscopy Adult female *P. persimilis* were placed in a fixative consisting of 1% paraformaldehyde and 1.5% glutaraldehyde in cacodylate buffer (pH 7.4) for 24 to 48 hr. The fixative was replaced with 0.12 M cacodylate buffer (pH 7.2) with three changes in 60 min. Tissue was postfixed for 2 hr in 1% osmium tetroxide in 0.12 M cacodylate buffer.

Following fixation, mites were placed in distilled water for 10 min and dehydrated in the following ethanol series: 50% (30 min), 70% (30 min), 90% (30 min), 100% (60 min), followed by propylene oxide:absolute ethanol (1:1) (30 min), propylene oxide (60 min), propylene oxide:low-viscosity Spurr resin (16 hr), and low-viscosity Spurr resin (24 hr). Each solution was changed three times. Each mite was placed in Spurr resin within a flat mould and cured for a minimum of 16 hr in a 60°C oven.

Ultrathin gold sections, approximately 100 nm thick, were cut with a diamond knife using an LKB Nova Ultramicrotome. Sections were placed on Formvar-coated grids and stained with prefiltered, 4% uranyl acetate for 20 min in a 60°C oven and then with lead citrate for 6 min at room temperature. Sections were examined with an Hitachi H-600 transmission electron microscope (acceleration voltage, 75 kV).

Results

Two unidentified species of microsporidia were found within *P. persimilis*, one each from the North American and Israeli sources. These are referred to as Species A and Species B, respectively. Although spores were detected, distinct developmental stages of these microsporidia were not observed in light microscopic examination of stained preparations. Life cycle observations and all measurements were obtained exclusively from transmission electron micrographs.

Species A (North American source)

Presporal stages Both specimens examined by TEM were infected with microsporidia. All stages observed with the electron microscope were uninucleate. The earliest stage observed was the schizont (Figure 5.1). Rounded-to-ovoid

schizonts measured 1.64±0.06 μm in width and 2.44±0.19 μm long (n=13). The nucleus (N) occupied a large portion of each schizont. Schizonts appeared to undergo cellular division to become groups of five, six or more cells (Figure 5.2). Several schizonts often occupied a single host cell and appeared to develop in direct contact with host cell cytoplasm (Figure 5.3). Schizonts were observed only within the cytoplasm of digestive cells. Sporonts could not be distinguished from schizonts.

Individual cells described here as early sporoblasts measured 2.73±0.48 x 1.49±0.15 μm (n=4). These cells often contained darkened nucleoli and were distinguishable by their electron-dense cell membrane and by the loosely-packed ribosomes that gave them a more granular appearance (Figure 5.4). These were proximal to mature spores and were observed within the cortex of the supraoesophageal ganglion, within cells underlying the cuticle and within eggs. One sporoblast was observed in cross-section within the nucleus of an unidentified cell type (Figure 5.5).

The mature spore Mature spores were elongate-ovoid (Figures 5.6 and 5.7) and measured 2.88±0.14 x 1.21±0.03 μm (n=11). A polar filament (PF) coiled seven to 10 times in the posterior half of the spore. In some cases, the final one to two coils appeared attenuated. The anchoring disc base (AB) was broad and flat and measured 277 nm, with stout arms (AA) each measuring approximately 188 to 222 nm in length (Figure 5.8). A lamellar polaroplast (P) occupied approximately one-third of the anterior region of the mature spore. Large cavities in the polaroplast are artifacts, probably resulting from incomplete fixation. Spores were densely packed with ribosomes and although posterior vacuoles were not commonly observed, they occupied one-third of the spore when present.

Spore walls were relatively uniform in thickness aside from a noticeable thinning at the anterior end. Measurements from micrographs showed them to be 78 to 200 nm thick and to consist of three layers: an innermost plasma membrane (PM), a translucent endospore layer (EN) measuring 44 to 167 nm, and an electron-dense exospore (E) measuring 16 to 33 nm (Figure 5.9).

Spores were occasionally observed within groups and did not appear to be

confined by an interfacial envelope. This, however, may be an artifact of poor fixation. Individual spores and groups of spores were observed throughout most infected tissues, including supra- and suboesophageal ganglia, cells lining the caecal lumen, muscle fibres, and cells underlying the cuticle. They were also observed in eggs within gravid females. Mature spores were not observed within digestive cells. Sporoblasts and spores were occasionally observed together.

Pathology Schizonts were observed within digestive cells, apparently in direct contact with cell cytoplasm. Sporoblasts and spores were found within the cytoplasm of cells lining the caecal lumen (Figure 5.10) and infected cells often hypertrophied (Figure 5.11). Sloughed cells containing microsporidian spores were observed within the caecal lumen. Spores were not present within digestive cells but were observed within all other infected tissues. Some tissues became packed with spores, including the cortex of the supra- and suboesophageal ganglia (Figure 5.12); in contrast, spores were rarely observed in the neuropile (Figure 5.13). Spores were also observed within cells underlying the cuticle (Figure 5.14), within muscle fibres (Figure 5.15), and in unidentified leg tissues (Figure 5.16). Spores were observed in eggs within gravid females (Figure 5.17).

Species B (Israeli source)

Five of six specimens examined were infected with microsporidia. Two of these were also infected with rickettsia (within unidentified tissues) while virus-like particles were observed in one specimen (within the yolk of eggs).

Presporal stages All stages observed with the electron microscope were uninucleate. Schizonts were generally more elongate than those of Species A (Figure 5.18) and measured 2.53±0.23 μm in width and 1.58±0.10 μm long (n=14). Variable numbers of schizonts were located within the nuclei of digestive cells and within the cytoplasm of cells lining the caecal lumen. When numerous, schizonts were observed within lightened areas of the host cell nucleus, suggesting that they these may be grouped together, although there was no evidence of interfacial envelopes (Figure 5.19). Sporonts could not be distinguished from schizonts.

Stages identified here as early sporoblasts contained a darkened nucleolus

and were distinguished from schizonts by a more electron-dense membrane and by loosely-packed ribosomes that gave them a more granular appearance (Figure 5.20). Early sporoblasts measured 2.06±0.19 x 1.41±0.09 μm (n=11) and were observed outside the cell membrane of digestive cells, within cells underlying the caecal lumen, and within groups of spores in unidentified tissue. Developing sporoblasts measured 2.88±0.19 x 1.12±0.03 μm (n=5). Each contained an immature polar filament and a prominent Golgi apparatus (Figures 5.21 and 5.22, G) that was less conspicuous in older sporoblasts (Figure 5.23). Developing sporoblasts were observed exclusively within the cytoplasm of cells lining the caecal lumen, proximal to mature spores.

The mature spore Mature spores were ovoid (Figure 5.24) and measured 2.65±0.23 x 1.21±0.07 μm (n=5). Although an isofilar polar filament coiled three to four times in the posterior half of the spore, densely packed ribosomes often concealed the polar filament and other internal spore structures. Features were more conspicuous in sporoblasts. The base of the anchoring disc was broad and measured 266 nm, with thin, short arms each measuring approximately 177 to 222 nm in length (Figure 5.25). A lamellar polaroplast occupied approximately one-third of the anterior region of the mature spore. Large cavities in the polaroplast are artifacts, probably resulting from incomplete fixation. Posterior vacuoles (PV) occupied one-third of the spore.

Spore walls were relatively uniform in thickness but thinner at the anterior end. Measurements from micrographs showed them to be 55-64 nm thick and consist of three layers: the innermost plasma membrane, a translucent endospore layer measuring 36-45 nm, and an electron-dense exospore measuring 9-18 nm (Figure 5.26).

Occasionally, host cells became packed with sporoblasts and spores (Figure 5.27). Groups of spores were occasionally observed within a dark matrix of unknown origin surrounded by a membrane described here as an interfacial envelope (Figure 5.28). Individual spores and groups of spores were observed within digestive cell nuclei, in both the nuclei and cytoplasm of cells lining the caecal lumen, in the supra- and suboesophageal ganglia, in cells underlying the

cuticle, and within cells of the Malpighian tubules. Spores were not observed within muscle fibres but were observed within the ovary and eggs of gravid females.

Pathology Nuclei of digestive cells containing few to numerous schizonts were greatly hypertrophied. Infected cells that sloughed into the caecal lumen contained developmental stages and mature spores in addition to food particles. Schizonts, sporoblasts and spores were observed within the cytoplasm of hypertrophied cells underlying the caecal lumen (Figure 5.29). Infected cells lining the Malpighian tubules hypertrophied (Figure 5.30), and numerous spores and presporal stages were observed within developing eggs and ovarian tissue (Figure 5.31).

Discussion

Presporal stages All stages of Species A, Species B and *M. phytoseiuli* were uninucleate. A summary of presporal characteristics for microsporidia found in *P. persimilis* is provided in Table 5.1. Schizonts of all three microsporidian species were observed within digestive cells and few to several schizonts were observed within an individual host cell. Round to ovoid schizonts of Species A (North America) developed within plasmodia-like groups, with each schizont sharing adjacent membranes. These were observed almost exclusively within digestive cell cytoplasm. Schizonts of Species B (Israel) appeared more elongate than did those of Species A, did not share adjacent membranes, and generally appeared independent of one another. Schizonts of species B favoured digestive cell nuclei for development. In contrast, round to slightly-ovoid schizonts of *M. phytoseiuli* (Europe) developed within digestive cell nuclei, typically within chromatin-rich areas adjacent to the nuclear membrane (see Chapter 4, Figures 4.11 and 4.15). Schizonts within cells lining the caecal lumen provide evidence that autoinfection occurred for Species B and *M. phytoseiuli*. Sporoblasts were not readily observed for Species A. Those of species B and *M. phytoseiuli*, however, were not restricted to specific tissues. Sporoblasts of *M. phytoseiuli* had a distinctive caudal prolongation extending from the posterior end (see Chapter 4, Figure 4.3); this structure was not observed in Species A or B.

Mature spores A summary of mature spore characteristics for microsporidia found in *P. persimilis* is provided in Table 5.1. Spores of Species A were elongate-ovoid and measured 2.88±0.14 x 1.21±0.03 μm. The polar filament coiled seven to 10 times within the posterior half of the spore. In some cases, the last two coils appeared attenuated. Spores of Species B were slightly smaller than those of Species A and measured 2.65±0.23 x 1.21±0.07 μm. Densely-packed ribosomes often concealed internal structures in the spores of Species B. When observed, an isofilar polar filament coiled two to four times in the posterior half of the spore. Therefore, similarities in spore size are likely to pose difficulties in distinguishing Species A and Species B by light microscopy. In contrast, *M. phytoseiuli* spores were larger than spores of the previous two species. They were broad- to elongate-ovoid and measured 4.33±0.35 x 1.27±0.15 μm in electron micrographs. An isofilar polar filament coiled 12 to 15 times in the posterior two-thirds of the spore (see Chapter 4; Figures 4.4 and 4.5).

Pathology A summary of pathology caused by microsporidia found within *P. persimilis* is provided in Table 5.2. Spores and developing stages of all three microsporidia were found in most *P. persimilis* tissues. The presence of numerous schizonts within digestive cells suggests that development begins in these cells. Schizonts from each species appeared to occupy distinct sites within these cells. Schizonts of Species A were within digestive cell cytoplasm while those of Species B and *M. phytoseiuli* were primarily located within digestive cell nuclei. For all three microsporidia, spores within cells that are sloughed into the caecal atrium likely provide the means for autoinfection.

In all cases, damage to the caecal wall was extensive. Infected caecal wall cells hypertrophied and often lacked microvilli. Damage to caecal wall cells suggested that mature spores were released into the caecal atrium. These were likely excreted with faeces onto foliage, and although they may have provided inoculum for horizontal transmission, the mechanism of transmission remains unknown. Spores within hypertrophied cells lining Malpighian tubules provided further evidence that spores of Species B were likely excreted. Excretion of spores, however, does not ensure that horizontal transmission will readily occur, as

illustrated for *M. phytoseiuli* in the laboratory (see Chapter 7). Although spores were found in faecal smears examined by light microscopy, examination of foliage surfaces by scanning electron microscopy revealed densely-packed aggregates of faecal material comprised primarily of dumbbell-shaped crystals (see Chapter 7). In this case, spores confined within faecal aggregates were not freely liberated onto foliage surfaces.

Numerous spores within the supra- and suboesophageal ganglion, muscle fibres and cells adjacent to the cuticle are unlikely to be horizontally transmitted to new hosts unless liberated into the environment during decomposition of the host body following death or when infected hosts are cannibalised. Despite having a less prominent role in horizontal transmission, microsporidian spores within these cells cause extensive damage to individual cells and to entire tissues. Spores or developmental stages observed throughout ovarian tissue (Species B) and within developing eggs suggest that all three microsporidia are transovarially transmitted. Vertical transmission has been confirmed in studies with live predators infected with *M. phytoseiuli* (see Chapter 7).

Alternative pathology If cells described herein as digestive cells are actually a portion of the lyrate organ, as proposed in Chapter 2, the pathological descriptions that precede would require modification. Regardless of the true identity of these cells, microsporidia were observed in remnants of cells within the caecal lumen of *P. persimilis* adult females. Early developmental stages of microsporidia in cells lining the caecal lumen and underlying muscle tissue provide evidence that autoinfection does occur.

According to Michael (1892), the lyrate organ should be clearly regarded as a portion of the ovary. The following alternative pathological description is based on the assumption that cells previously described herein as digestive cells in *P. persimilis* are actually cells of the lyrate organ. Early stages of all three microsporidia reported in *P. persimilis* appeared to occupy distinct sites within these cells. Schizonts of Species A were within the cell cytoplasm while those of Species B and *M. phytoseiuli* were primarily located within the nuclei of these cells. Regardless of the location of the schizonts, infection of these cells suggest a

means to contribute toward the infection of developing eggs within the gravid female, and thereby ensure that transovarial transmission does occur.

The function of the lyrate organ is unclear. Michael (1892) suggested that it may have either a germiniferous or vitelligenic function. The presence of numerous vesicles within these cells suggest that they likely function as a vitelligenic organ and are therefore involved in loading yolk into the rapidly maturing eggs. If these cells are infected with microsporidia, parasites are likely introduced into the maturing egg along with the yolk.

Internal morphology and tissue descriptions of *P. persimilis* were based solely on the examination of adult female predators. Further work is required to determine if cells of the proposed lyrate organ are restricted to the adult female or whether these cells are present in earlier developmental stages of *P. persimilis*. According to Michael (1892), the lyrate organ was present in *Haemogamasus horridus* nymphs, albeit very small and although the lyrate organ appeared to be much larger in the adult female, this organ became its largest in ovipositional females. The lyrate organ of adult female *P. persimilis* infected with *M. phytoseiuli* appears to be moderately to heavily infected with early developmental stages of the pathogen. Based on this observation, it seems reasonable to conclude that this organ becomes infected prior to adulthood. Although the internal anatomy of immature *P. persimilis* was not investigated during this study, it is likely that the lyrate organ is present in immature *P. persimilis*.

Distinct species vs. spore dimorphism Spore dimorphism is common among microsporidia from many hosts. In mosquito hosts, heterosporous microsporidia produce more than one spore type, each of which is responsible for a specific type of transmission (Garcia *et al.*, 1993; Hazard and Fukuda, 1974). Spore dimorphism has also been reported for the microsporidium *Nosema muscidifuracis* Becnel and Geden in *Muscidifurax raptor* Girault and Saunders, a parasitoid used to control muscoid flies. In this case, the microsporidium produces diplokaryotic spores with three distinct morphologies, each believed to be responsible for a specific mode of transmission. One spore type is thought to be responsible for autoinfection, the second for horizontal transmission, and the third, found within

eggs of *M. raptor*, for either initiation and spread of infection at eclosion or horizontal infection to a new host when eggs are cannibalised (Becnel and Geden, 1994).

Spores of Species A were found only within *P. persimilis* from North America. Those of Species B were found within predators from Israel, and spores of *M. phytoseiuli* were observed only within predators from a European source. *Microsporidium phytoseiuli* spores were much larger than those of Species A or Species B and the polar filament coiled 12 to 15 times within the posterior two-thirds of the spore. Sporoblasts had a unique caudal prolongation not observed in sporoblasts of the other species. Spores of Species A and B were similar in size and shape, but ultrastructurally distinct. The polar filament coiled seven to 10 times within the posterior half of spores of Species A, the last two coils occasionally appearing attenuated. In contrast, an isofilar polar filament coiled two to four times in the posterior half of spores of Species B (Table 5.1). Furthermore, schizonts of all three species appeared to have a site preference for development within digestive cells of the host (Table 5.2). Based on spore ultrastructure, pathological observations and the exclusive origin of each microsporidium observed, these likely represent three distinct microsporidian species rather than the spore dimorphism of a single species.

Invasion of host cell nuclei Although host cell nuclei are rarely invaded by microsporidia (Canning, 1990), several exceptions have been observed. *Nosema apis* (in the honeybee, *Apis mellifera*), *N. fumiferanae* (in spruce budworm, *Choristoneura fumiferana*) and *N. bombycis* (in the silkworm, *Bombyx mori*) have been observed within cell nuclei of their respective hosts (Tanada and Kaya, 1993). *Nosema* sp. were detected in the nuclei and cytoplasm of midgut muscle cells in the parasitic wasp *Pediobius foveolatus* (see Chapman and Hooker, 1992). *Steinhausia mytilovum*, a microsporidium infecting nurse cells of ovaries and oenocytes of the mussel, *Mytilus edulis*, occasionally infect host cell nuclei (Sprague *et al.*, 1992). *Enterocytozoon salmonis* and *Microsporidium rhabdophilia* develop exclusively within cell nuclei of salmonid fish (Chilmonczyk *et al.*, 1991).

For all three microsporidian species within *P. persimilis*, at least one

developmental stage was observed within cell nuclei. A single sporoblast of Species A was observed within the nucleus of an unidentified cell type, whereas schizonts of both Species B and *M. phytoseiuli* appeared almost exclusively in the nuclei of digestive cells.

Host specificity Microsporidia, once considered host specific, often were identified on the basis of the hosts they infected. Microsporidia have since been found to infect a wide range of hosts and generalisations are no longer made regarding host specificity. Members of different orders of insects may be susceptible to the same microsporidium, as is commonly observed when a hymenopterous parasitoid becomes infected with the same microsporidian species that infects its lepidopteran host (Brooks, 1973). Furthermore, a single species of microsporidia may infect animals belonging to different classes, as is the case for microsporidia that infect mosquitoes as well as secondary copepod hosts (see Tanada and Kaya, 1993).

Current literature is dominated by ultrastructural descriptions of microsporidian pathogens, and information regarding the number of pathogenic species within a single host are rare. Recent changes in microsporidian taxonomy and studies reporting additional ultrastructural features of some microsporidia often have resulted in the reclassification of microsporidian genera or species. Due to these changes, it is difficult, in some cases, to determine whether a single host is infected by one or by several species of microsporidia. For example, a single microsporidian species reported in *Apanteles glomeratus* L., a parasitoid of the cabbageworm *Pieris brassicae* L., was first described as *Perezia legeri* Paillot (Paillot, 1918). According to Hostounský (1970), this microsporidium was subsequently described as *Perezia mesnili* Paillot (Tanada, 1955) and *Nosema polyvora* Blunck (Blunck, 1954; Issi and Maslennikova, 1966), before they were recognised as a single species and renamed *Nosema mesnili* Paillot (Hostounský, 1970).

A general bias toward the investigation of pathogens within insects of agricultural or veterinary importance has resulted in limited information about host specificity from a select group of arthropods. For example, previous studies

regarding microsporidioses of beneficial insects have described only four reports of multiple infections. *Campoletidis sonorensis* Cameron, a hymenopterous parasitoid of corn earworm, *Heliothis zea* Boddie, is host to two microsporidian species, *Nosema heliothidis* Lutz and Splendor and *N. campoletidis* Brooks and Cranford (Brooks and Cranford, 1972). These microsporidia also infect the hyperparasitoids *Catolaccus aenoviridis* Girault and *Spilochalcis side* Walker (McNeil and Brooks, 1974). Cossentine and Lewis (1988) reported two species of *Nosema* in *Lydella thompsoni*, a tachinid parasitoid of the European corn borer, *Ostrinia nubilalis*. Beerling *et al.* (1993) reported three species of undescribed microsporidia in mass rearings of *Amblyseius cucumeris* and *A. barkeri*, two acarine predators used for control of thrips in commercial greenhouses. Based on the frequency of these reports, the occurrence of more than one microsporidian species within a single arthropod host would appear to be uncommon. Thus, the description of three species of microsporidia within one host species, *P. persimilis*, seems unusual and is quite intriguing.

Microsporidia reported from other mites Microsporidia previously reported from terrestrial and aquatic mites are listed in Chapter 4 (see Table 4.1). Of these, only two microsporidian species from terrestrial mites have dimensions similar to those reported for Species A and B from *P. persimilis*. Spores of *Nosema steinhausi*, a microsporidium infecting the forage mite *Tyrophagus noxius*, measure 2.2-3 x 1-1.5 μm but have paired nuclei during development (Weiser, 1956). Spores of the microsporidium *Pleistophora oribatei* Purrini and Weiser, described from oribatid mites, measure 2.0-2.5 x 1.0-1.5 μm and are located within lymphocytes, oenocytes, gonads and the gut wall (Purrini and Weiser, 1981). Although the description of *P. oribatei* is brief and ultrastructural observations are lacking for both microsporidian species, differences in morphology and pathology suggest that Species A and Species B likely represent two previously unreported microsporidian species.

Phytoseiulus persimilis and microsporidia *Phytoseiulus persimilis* was first discovered in 1960, when it was inadvertently imported from Chile to Germany in a shipment of orchids (Hussey, 1985). This predator has since been mass-

produced and distributed throughout the world for release on a variety of greenhouse and field crops.

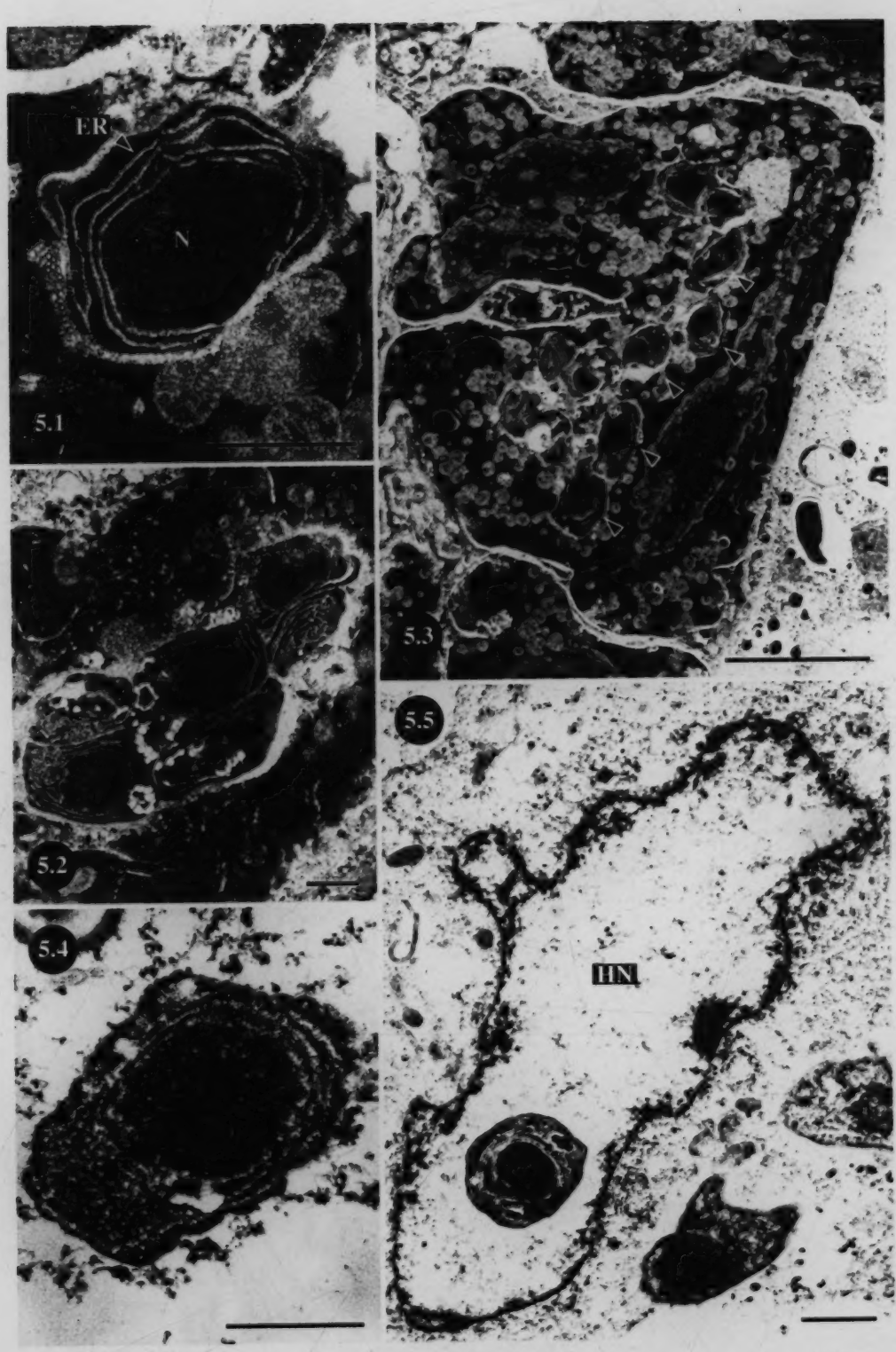
The discovery of three microsporidian species in *P. persimilis* almost 40 years following the initial discovery of this predator raises questions regarding the source of these pathogens. Since distinct microsporidian species were observed in *P. persimilis* obtained from three different locations, it seems unlikely that these pathogens originated within Chile and were then distributed to different areas of the world. Even if this were the case, subsequent predator collection from the field or restocking of predators from existing commercial or research sources would likely contribute toward the distribution or redistribution of these pathogens.

Although the sources of these microsporidia are unknown, it seems more reasonable to conclude that these microsporidia are endemic to the regions in which *P. persimilis* are mass-produced. Although microsporidian spores were not found in *Tetranychus urticae* during my study, it is possible that prey mites from the original sources were also infected with the microsporidia described herein. Prey mites from these sources, however, were not available for study. It is also possible that the microsporidian pathogens of other endemic acarine species may infect *P. persimilis*.

Figures 5.1-5.5. Presporal stages of the microsporidium found infecting *P. persimilis* from North America (Species A).

Figure 5.1. Schizont.
Figure 5.2. Group of six schizonts.
Figure 5.3. Several schizonts (arrowheads) within cytoplasm of a single digestive cell.
Figure 5.4. Early sporoblast.
Figure 5.5. Sporoblast within nucleus of unidentified cell.

Scale bars: Figures 5.1, 5.2, 5.4, and 5.5, 1 μm; Figure 5.3, 5 μm.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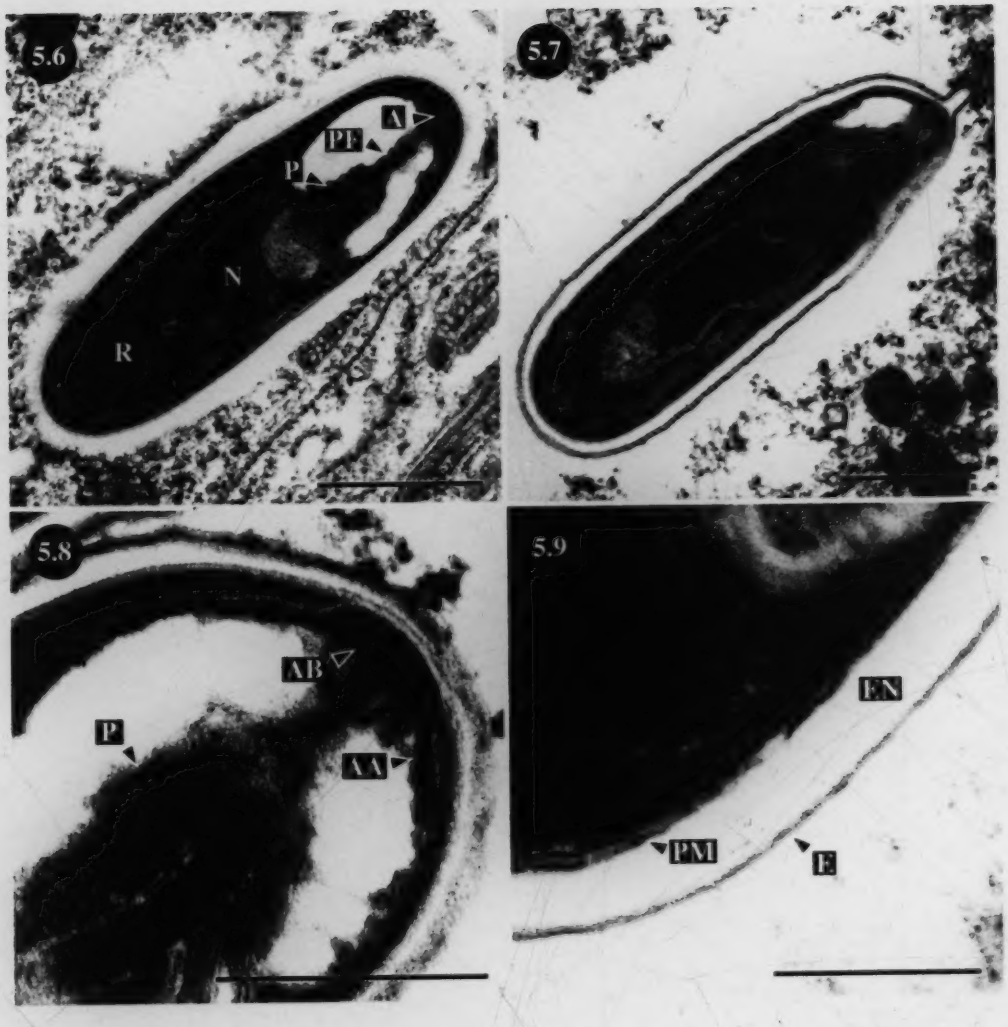
Figures 5.6-5.9. Mature spores and spore characteristics of the microsporidium found infecting *P. persimilis* from North America (Species A).

Figure 5.6. Mature spore with anisofilar polar filament.
Figure 5.7. Mature spore. Spore wall distortion at anterior end is an artifact.
Figure 5.8. Anchoring disc detail.
Figure 5.9. Spore wall.

Scale bars: Figures 5.6 and 5.7, 1 μm; Figures 5.8 and 5.9, 0.5 μm.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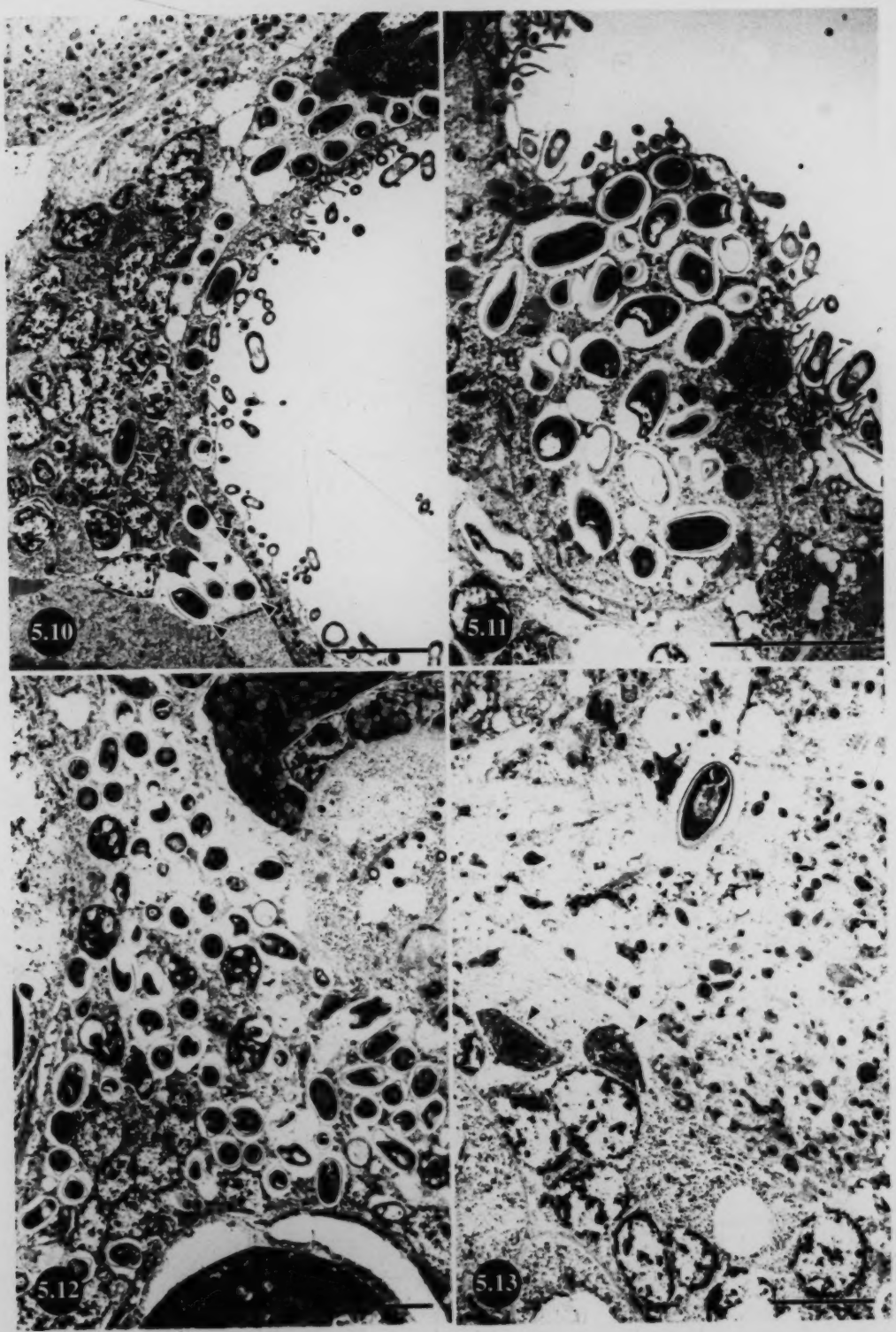
Figures 5.10-5.13. Pathology caused by the microsporidium found infecting *P. persimilis* from North America (Species A).

Figure 5.10. Sporoblast and spores within cells lining the caecal lumen and adjacent cortex of supraoesophageal ganglion (arrowheads).
Figure 5.11. Hypertrophy of caecal wall cell packed with microsporidian spores.
Figure 5.12. Numerous spores packed within cortex of supraoesophageal ganglion.
Figure 5.13. Infection of supraoesophageal ganglion; spore within neuropile and sporoblasts (arrowheads) within cortex.

Scale bars: 5 μm.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Figures 5.14-5.17. Pathology caused by the microsporidium found infecting *P. persimilis* from North America (Species A).

Figure 5.14. Mature spores within cells underlying the cuticle.
Figure 5.15. Mature spore within muscle fibres.
Figure 5.16. Spores and evacuated spore (arrowheads) within leg tissues.
Figure 5.17. Sporoblast (arrowhead) and spores in eggs within gravid females.

Scale bars: 5 μm

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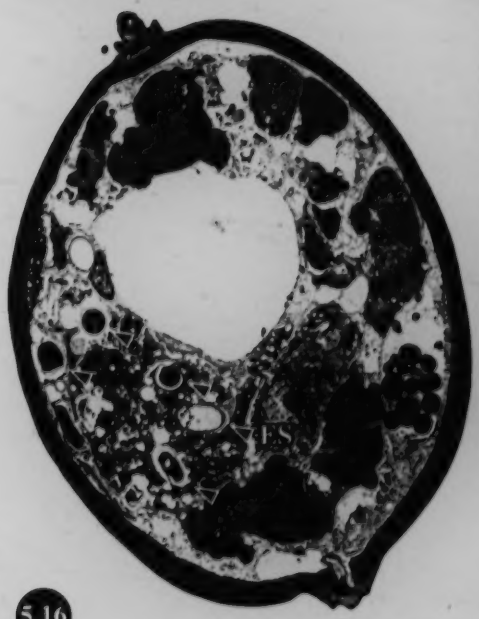

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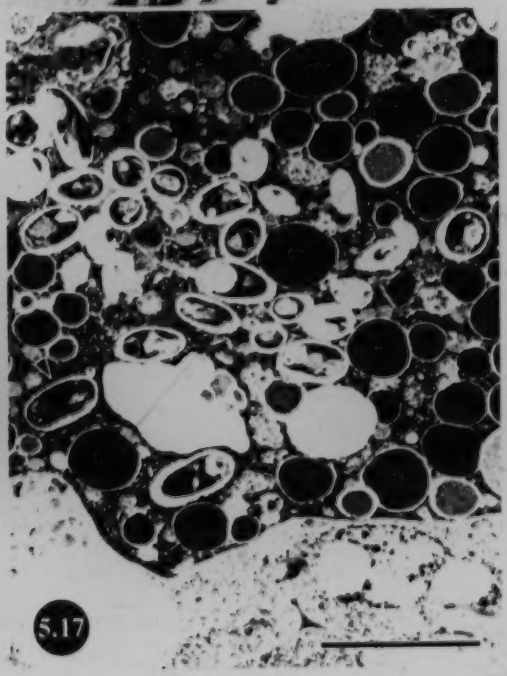
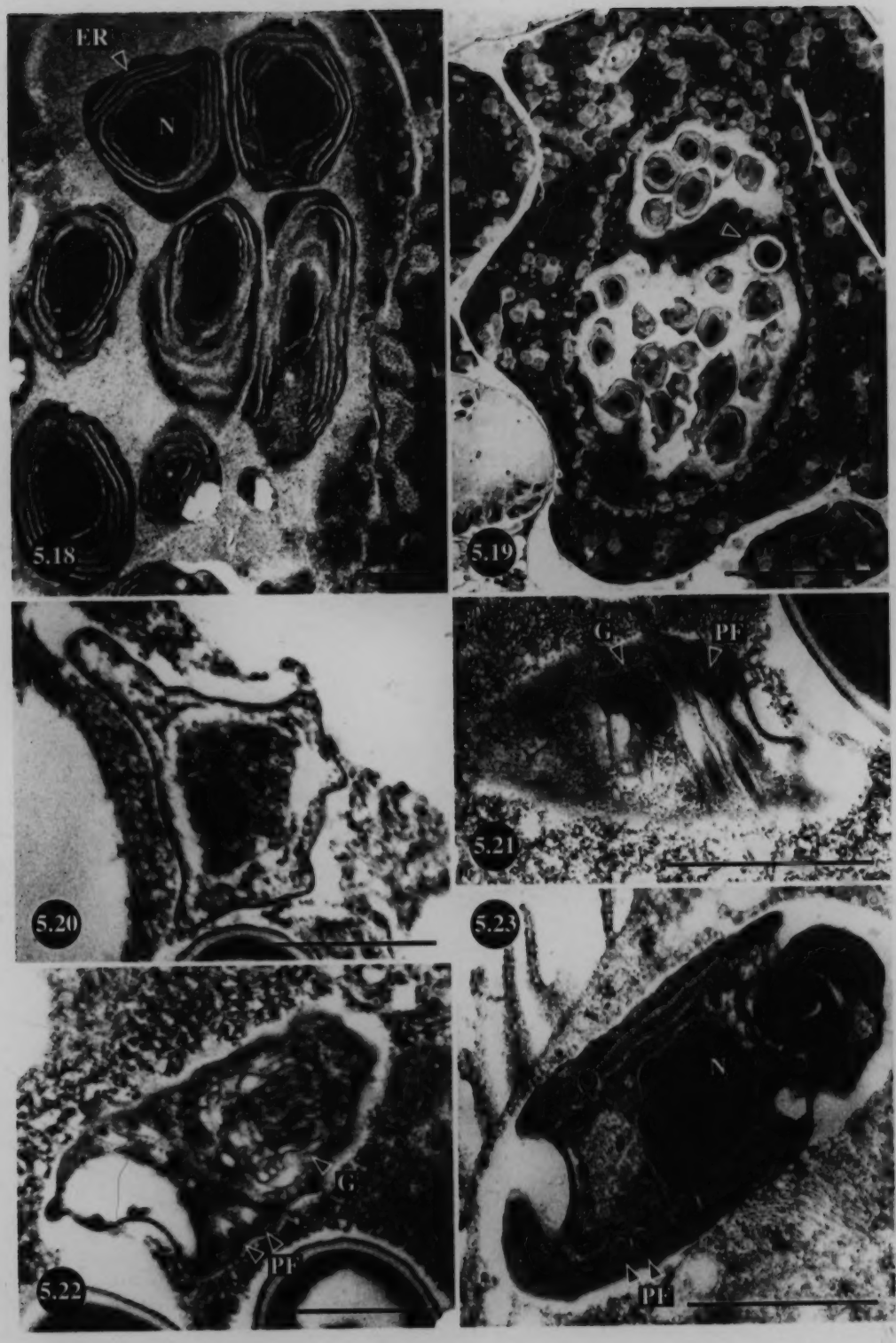


5.17

Figures 5.18-5.23. Presporal stages of the microsporidium found infecting *P. persimilis* from Israel (Species B).

Figure 5.18. Schizonts in digestive cell nucleus.
Figure 5.19. Numerous schizonts and mature spore (arrowhead) within digestive cell nucleus.
Figure 5.20. Early sporoblast.
Figure 5.21. Early sporoblast development showing development of the polar filament and prominent Golgi apparatus.
Figure 5.22. Early sporoblast development.
Figure 5.23. Sporoblast.

Scale bars: Figures 5.18, 5.20, 5.21 5.22 and 5.23, 1 μm; Figure 5.19, 5 μm.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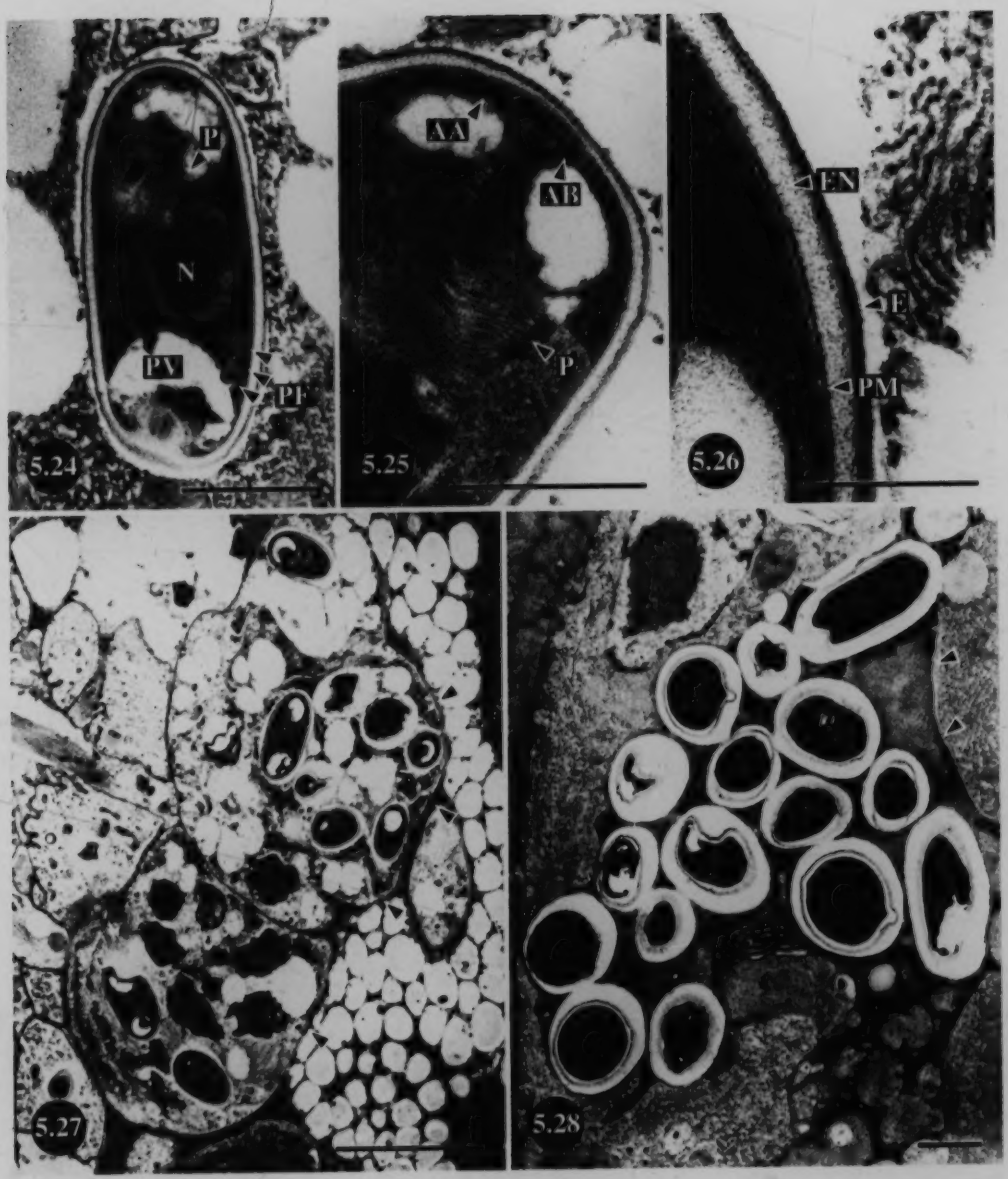
Figures 5.24-5.28. Mature spores and spore characteristics of the microsporidium found infecting *P. persimilis* from Israel (Species B).

Figure 5.24. Mature spore.
Figure 5.25. Anchoring disc and polaroplast detail.
Figure 5.26. Spore wall.
Figure 5.27. Sporoblasts and spores packed within host cell membranes (arrowheads).
Figure 5.28. Group of mature spores within a darkened matrix and surrounded by a membrane of unknown origin (arrowheads).

Scale bars: Figures 5.24, 5.25, and 5.28, 1 μm; Figure 5.26, 0.25 μm; Figure 5.27, 5 μm.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Figures 5.29-5.31. Pathology caused by the microsporidium found infecting *P. persimilis* from Israel (Species B).

Figure 5.29. Schizonts (arrowheads), sporoblasts and mature spores within cells lining the caecal lumen.

Figure 5.30. Infected cells of Malpighian tubules causes cell hypertrophy. Inset: Spores within infected cells are sloughed into the Malpighian tubules.

Figure 5.31. Sporoblasts within egg (arrowhead, upper middle) and ovary (lower centre). Numerous spores within ovarian tissue and young, developing egg (arrowhead, middle right).

Scale bars: Figures 5.29 and 5.30, 5 μm; Figure 5.30 inset, 1 μm. Figure 5.31, 10 μm.

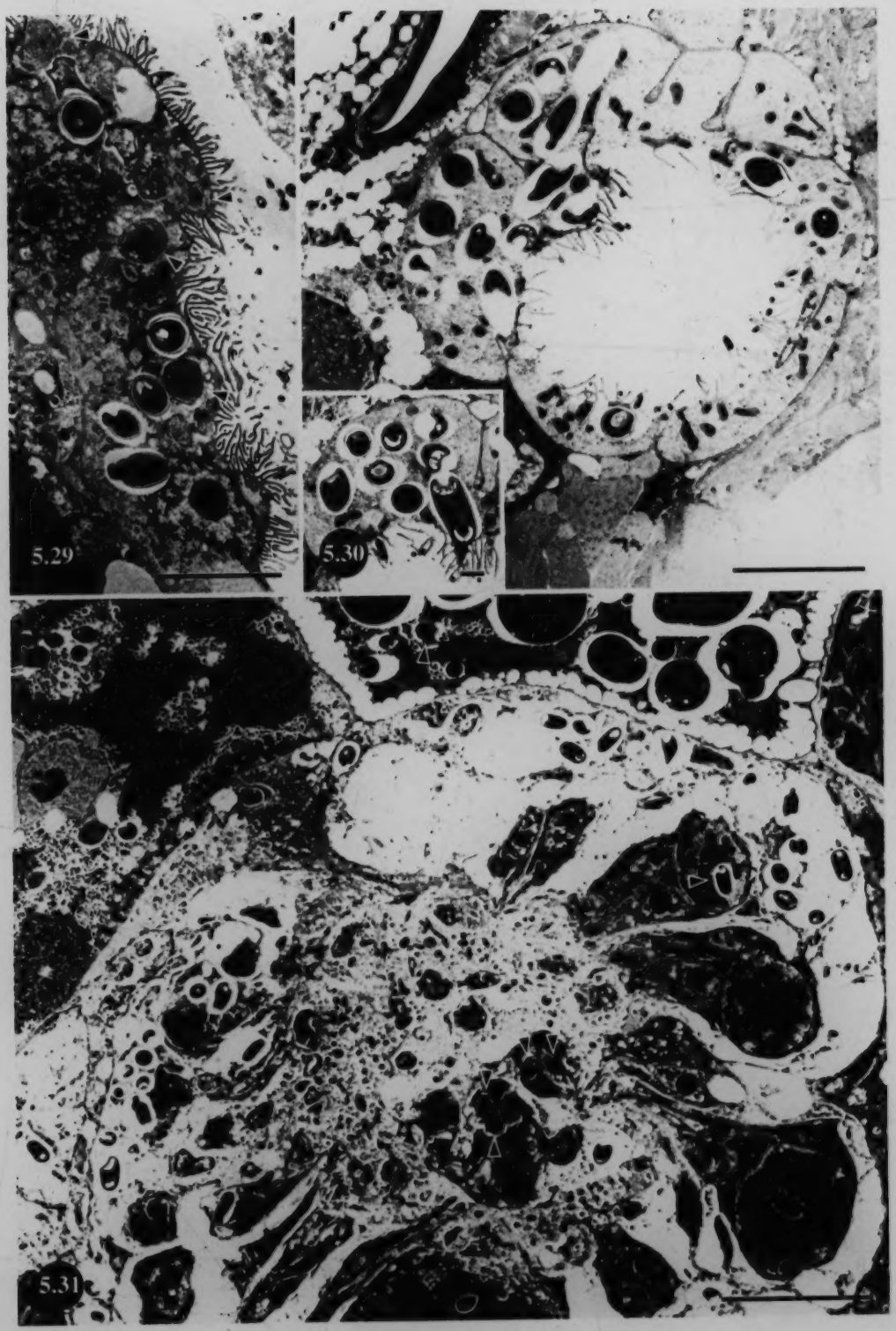
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Table 5.1
Summary of Presporal and Mature Spore Characteristics of Microsporidia Found in *Phytoseiulus persimilis* from Three Commercial Sources

	Unidentified Species A (North America)	Unidentified Species B (Israel)	*Microsporidium phytoseiuli* (Europe)
Presporal stages			
Schizonts	single or groups of 5 or more	variable numbers of schizonts	single or groups of 2 or more
Schizont measurement (μm)	2.44±0.19 x 1.64±0.06	2.53±0.23 x 1.58 ± 0.10	1.9 - 2.9
Sporoblasts	one observed in cross section	numerous; in cytoplasm of caecal wall cells	common; caudal prolongation
Sporoblast measurement (μm)	none available	2.88±0.19 x 1.12±0.03	up to 5.6 μm long; similar size as spores
Mature Spore			
Measurement (μm)	2.88±0.14 x 1.21±0.03	2.65±0.23 x 1.21±0.07	4.33±0.35 x 1.27±0.15
Polar filament	anisofilar/isofilar	isofilar; polar filament often concealed by densely-packed ribosomes	isofilar
No. of polar filament coils	7 to 10; coiled in posterior 1/2 of spore	2 to 4; coiled in posterior 1/2 of spore	12 to 15; coiled in posterior 2/3 of spore
Polaroplast	lamellar; occupied 1/3 anterior region	lamellar; occupied 1/3 anterior region	lamellar; occupied 1/3 anterior region
Anchoring disc base/arms (nm)	277/188-222	266/177-222	150/250
Spore wall (nm)	78-200	55-64	89-101
Endospore/ exospore (nm)	44-167/16-33	36-45/9-18	54-65/30-36
Posterior vacuole	uncommon; occupied 1/3 of spore if present	occupied 1/3 of spore	occupied 1/4 of spore
Interfacial envelopes	none observed	spores occasionally surrounded by membrane of unknown origin	present; spores in groups of 4,8,16 or more

Table 5.2
Summary of Pathology Caused by Microsporidia in *Phytoseiulus persimilis* from Three Commercial Sources

	Unidentified Species A (North America)	Unidentified Species B (Israel)	*Microsporidium phytoseiuli* (Europe)
Tissue Tropism			
Digestive cells (Lyrate organ)	few to several schizonts within cell cytoplasm	few to numerous schizonts primarily within lightened areas of cell nuclei and occasionally cell cytoplasm; spores in cell nuclei	few to several schizonts within chromatin-rich area of cell nuclei (adjacent to nuclear membrane); schizonts in cell cytoplasm; spores and sporoblasts in cell cytoplasm; spores in cell nuclei
Caecal wall cells	sporoblasts, spores in cell cytoplasm	schizonts, sporoblasts and spores within cytoplasm; spores in nuclei	schizonts, sporoblasts and spores in cytoplasm
Supra- and suboesophageal ganglia	spores rarely in neuropile; spores and sporoblasts in cortex	spores in cortex only	spores in cortex only
Cells underlying cuticle	sporoblasts, spores in cytoplasm	schizonts, spores in cytoplasm	spores in cytoplasm
Muscle fibres	spores in cytoplasm (within fibres)	tissue not observed	spores in cytoplasm; schizonts in cytoplasm of muscle underlying caecal wall
Cells lining Malpighian tubules	tissue not observed	spores in cytoplasm	tissue not observed
Eggs[†]	sporoblasts, spores	schizonts, spores	spores
Ovarian tissue	tissue not observed	schizonts, sporoblasts and spores	tissue not observed

[†] eggs observed within gravid females.

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Chapter 6

Effects of *Microsporidium phytoseiuli* (Protozoa: Microspora) on Performance of the Predatory Mite, *Phytoseiulus persimilis* (Acari: Phytoseiidae)

Introduction

Microsporidia, the most common protozoan pathogens of insects, infect both beneficial and pest arthropods. Although acute microsporidiosis may result in premature death, chronic infections are thought to be responsible for the regulation of some insect populations (Kluge and Caldwell, 1992; Tanada and Kaya, 1993). Chronic microsporidioses may either reduce the efficacy of arthropods used for biological control or interfere with the establishment of mass rearings (Kluge and Caldwell, 1992). Regardless of the type of infection produced, the occurrence of microsporidia in commercially produced arthropods is undesirable.

Recent reports of microsporidioses in mass-reared predatory mites *Neoseiulus cucumeris* (formerly *Amblyseius*), *Amblyseius barkeri* and *Phytoseiulus persimilis* have raised doubts regarding the quality of commercially produced biological control agents (Beerling and van der Geest, 1991; Steiner, 1993; Bjørnson *et al.*, 1996). Reports of pathogens and concerns regarding their performance have resulted in recommendations for quality control guidelines for more than 20 greenhouse biological control agents (van Lenteren, 1994). Although proposed tests are designed to ensure minimum performance standards are achieved, current recommendations do not promote routine examination of biological control agents for pathogens, nor do guidelines consider the effects that pathogens may have on the performance of natural enemies. Without consideration of pathogens and their effects on biological control agents, it remains uncertain if recommended performance parameters are reliable indicators of quality.

Routine examination of commercially produced *P. persimilis* resulted in detection of *Microsporidium phytoseiuli* in predators from a European source. The ultrastructure and pathology of this microsporidian pathogen have been described (Bjørnson *et al.*, 1996; also Chapter 4 in this thesis). The aim of this study has been to quantify the effects of *M. phytoseiuli* on the performance of *P. persimilis* in

terms of short-term oviposition and survival, lifetime oviposition and longevity, sex ratio, and prey consumption. The results of short-term performance tests were compared to the expected results outlined in current quality control guidelines for *P. persimilis* (see van Lenteren, 1994). When possible, results were compared to those from uninfected predators that were from the same European source and had been received in the same shipment as those infected with *M. phytoseiuli*. These predators had been previously culled from infected predators and reared as an uninfected colony under quarantine conditions. Despite attempts to maintain a disease-free status in this colony, all predators eventually became infected with microsporidia. Once this occurred, performance of infected predators were then compared to uninfected predators obtained from two other commercial sources, one each from North America and Europe.

Materials and Methods

Mite rearing *Phytoseiulus persimilis* used initially in this study were obtained from a commercial insectary in Europe (source EU1) in December 1993 and reared at the University of Alberta (Edmonton, AB). Predators were maintained on *Tetranychus urticae*-infested bean plants (*Phaseolus vulgaris*) in cages that were placed within a controlled environmental chamber (16L:8D; 25°C:20°C). Each cage contained two spider mite-infested plants. Predators were fed twice each week by replacing one of the caged plants with a fresh, spider mite-infested plant. Used pots and soil were discarded.

The original shipment of *P. persimilis* from source EU1 consisted of both uninfected and microsporidia-infected predators. Because vertical transmission of the microsporidium was 100 percent (see Chapter 7), uninfected predators could be distinguished from infected ones by examining eggs produced by randomly selected gravid females for microsporidian spores. Uninfected or microsporidia-infected predators were used to establish separate colonies and each was isolated and maintained under quarantine conditions.

Predators from source EU1 were examined by transmission electron microscopy and no other pathogens were detected. Individual predators were routinely removed from each colony and examined for microsporidian spores.

The colony established from uninfected predators was maintained pathogen-free until June 1996. Uninfected predators from source EU1 were used to evaluate the short-term performance of *P. persimilis*. All individuals from this predator colony, however, had become infected with microsporidia by the time lifetime performance was to be evaluated. Therefore, *P. persimilis* obtained from a second European source (EU2) were used to evaluate lifetime performance. Predators from source EU2 and from a North American source (NA) were also evaluated for short-term oviposition and survivability.

Observation dishes Observation dishes used to study predator performance were constructed from 54 mm diameter Millipore dishes (see Appendix 4). A 30 mm diameter hole was cut in each dish base and covered with 90 μm nylon screen to permit air movement. Each lid was lined with a moistened 50 mm diameter disc cut from Filterbond landscape fabric to prevent desiccation of excised leaf discs. Fabric discs were soaked in 10% bleach solution for 20 min, then rinsed in sterile water before use.

Excised leaf discs, 27 mm in diameter, were cut from spider mite-infested bean leaves and placed on moistened fabric discs, with the upper leaf surface in contact with the fabric. Dishes were inverted, screen side facing downward, to simulate the leaf's natural orientation. Observation dishes were placed in a large plastic container within an incubator (16L:8D; 25°C:20°C). Relative humidity within the incubator was 70±10%; relative humidity within dishes was not measured.

Short-term performance

Performance of *P. persimilis* was evaluated for seven day test periods. For all performance studies, gravid *P. persimilis* females were randomly selected, placed in dishes and given 24 hr to adapt to test conditions before data were collected. Leaf discs were replaced every four to five days during all studies. Data were collected over six days and analyzed; data also was analyzed for the first five days of each study period so that performance values could be compared directly to those outlined in current quality control guidelines (van Lenteren, 1994).

Short-term oviposition Uninfected and microsporidia-infected *P. persimilis* females were randomly selected and isolated on spider mite-infested leaf discs within observation dishes. The numbers of eggs laid by predatory mites were recorded every 24 hr. Eggs laid on the first day were discarded; eggs laid from day 2 to day 7 were used for analysis. Mean daily oviposition rates (eggs per female per day) were calculated over five days (days 2 to 6 of the study) for comparison to results of previous studies and over six days (days 2 to 7). Each female was smeared upon death or on the seventh day of the study to determine infection status.

Since unmated females do not produce eggs (Laing, 1968; Scopes, 1985), the females that failed to produce eggs during study periods were not included in data analysis. The only exceptions were females that produced eggs during the 24 hour adjustment period prior to data collection but failed to produce eggs during the data collection period or those that were gravid when dissected after death. For all oviposition and prey consumption studies, a two-tailed t-test was used to determine significance between grand means.

Short-term survival Longevity of all female predators was recorded as percent survival over five days (days 2 to 6) and six days (days 2 to 7).

Prey consumption Leaves from spider mite-infested plants were removed and washed over a series of four sieves that varied in mesh size. Sieves were positioned so the bottom sieve had openings of 90 μm. Prey mites and eggs collected in the final sieve were transferred onto filter paper and gently dried by vacuum-filtration using a Buchner funnel. Forty live prey deuteronymphs were removed from the filter paper with a fine bristle paint brush and prey were placed on leaf discs cut from the leaves of mite-free plants. Infected and uninfected adult female predators were randomly selected and each isolated within an observation dish. Prey consumption was measured as the number of deuteronymphs eaten, observed as shrivelled cadavers that remained within the observation dish. Cadavers were counted and removed every 24 hr and replaced with live prey deuteronymphs. Prey consumption rates were calculated as mean deuteronymph prey eaten per female per day over five and six days.

Short-term performance of P. persimilis from two commercial sources After uninfected predators from source EU1 became infected with microsporidia, predators were obtained from two commercial sources, one each from Europe (EU2) and North America (NA). Colonies were established from single shipments of 1000 predators and maintained under controlled conditions as previously described. Predators from these colonies were routinely examined for microsporidian spores.

Predators from sources EU2 and NA were evaluated for short-term oviposition and survivability 10 and 30 days after arrival, then compared to those known to be infected with microsporidia (EU1). For all studies, gravid female predators from all sources were randomly selected and examined for microsporidian spores upon death or at the end of the study period. Predators from source EU2 were later used in lifetime performance studies.

Sex ratio Ninety-four eggs collected from both uninfected (EU2) and microsporidia-infected (EU1) females were isolated within observation dishes. Sex was determined on the fifth day following egg isolation; identification was based on size differences. Females are considerably larger than males at this stage. A 2x2 Chi-square contingency table was used to determine significance between sex ratios (Bailey, 1981).

Lifetime performance

Lifetime oviposition and longevity Data acquired during a study to determine parental contribution toward the infection of progeny (see Chapter 7) was pooled to determine the lifetime oviposition and longevity of both uninfected (source EU2; n=33) and microsporidia-infected female predators (source EU1; n=36). During the above experiment, females from the two sources were mated with both uninfected and microsporidia-infected males; however, there was no significant difference between the number of eggs produced by uninfected females, regardless of the infection status of the male (see Table 7.1). Similarly, infection status of the male had no effect on the mean number of eggs produced by microsporidia-infected females.

Uninfected and microsporidia-infected gravid females were isolated for 24

hours within observation dishes and eggs produced during this period were removed. Each egg was isolated in an observation dish and parent females were smeared to determine infection status. Eggs were permitted to hatch and progeny were sexed. On the seventh day of development, virgin females were mated by placing a single, virgin male (either uninfected or infected) within each dish for 24 hr. Uninfected and microsporidia-infected females were observed throughout their lifetimes. Female *P. persimilis* produce eggs only after mating has occurred (Laing, 1968); therefore, only female *P. persimilis* that produced eggs were used this study. Egg production was recorded daily and all females were examined for pathogens upon death.

A two-tailed t-test was used to determine the significance between means and grand means generated from oviposition and longevity studies, respectively. Age-specific survival and oviposition rates were calculated. Data were used to generate age-specific survival and oviposition curves where Day 0 represents the seventh day of development of females (when mating occurred).

Results

Short-term performance

Short-term oviposition Short-term oviposition rates and survivability of uninfected and microsporidia-infected *P. persimilis* from the same European source (EU1) are reported in Table 6.1. Predators infected with microsporidia produced significantly fewer eggs than uninfected females ($P<0.005$). Mean daily oviposition rates over five days were 2.63 ± 0.24 and 1.72 ± 0.18 eggs per female per day for uninfected and infected females, respectively. Mean daily oviposition rates were slightly lower when calculated over six days, as uninfected and microsporidia-infected females produced 2.60 ± 0.23 and 1.66 ± 0.18 eggs per female per day, respectively. Uninfected predators ($n=46$) produced a total of 706 eggs over six days of the study, whereas 521 eggs were produced by infected *P. persimilis* ($n=51$). Some infected predators appeared gravid but produced few or no eggs while remaining alive for several days.

Oviposition rates recorded during prey consumption studies were similar to oviposition rates observed during short-term oviposition tests. Mean daily

oviposition rates calculated over five days were 2.53±0.13 and 1.61±0.20 eggs per female per day for uninfected and infected mites, respectively (Table 6.1). Predators infected with microsporidia produced significantly fewer eggs than uninfected females ($P<0.002$). Mean daily oviposition rates were slightly lower when calculated over six days. Uninfected and microsporidia-infected *P. persimilis* females produced 2.38±0.13 and 1.50±0.19 eggs per female per day, respectively. Uninfected predators (n=34) produced a total of 523 eggs over six days during prey consumption studies, while 352 eggs were produced by microsporidia-infected predators (n=33).

Short-term survival Percent survival (over five days) was highly variable and ranged from 58.7 to 91.2 percent for uninfected mites (source EU1) and 60.8 to 72.7 percent for mites infected with microsporidia (Table 6.1).

Prey consumption Prey consumption rates for both uninfected and microsporidia-infected *P. persimilis* from source EU1 are reported in Table 6.2. Microsporidia-infected predators consumed significantly fewer prey than uninfected mites ($P<0.002$). Mean prey consumption over five days was 18.74±1.01 and 13.39±1.20 deuteronymph *T. urticae* per female per day for uninfected and infected predators, respectively. Over six days, mean prey consumption decreased slightly to 17.85±0.96 deuteronymphs per female per day for uninfected mites and 13.02±1.15 for mites infected with microsporidia. Uninfected predators (n=34) consumed a total of 3670 *T. urticae* deuteronymphs over six days, whereas microsporidia-infected predators (n=33) consumed 2812 prey.

Short-term performance of P. persimilis from two commercial sources Mean daily oviposition rates and percent survival of uninfected predators from a North American (NA) and second European (EU2) source were evaluated against microsporidia-infected mites from source EU1. Although no uninfected predators from source EU1 were available for performance evaluation at the time of these studies, data regarding performance of uninfected predators from source EU1 from an earlier study have been included in tables for comparison.

North America Predators from the North American insectary produced

significantly fewer eggs than microsporidia-infected predators from source EU1 ($P<0.001$) (Table 6.3). When evaluated for performance 10 days after arrival, *P. persimilis* from North America produced 1.06 ± 0.19 eggs per female per day over a five day period, whereas microsporidia-infected predators (EU1) produced 2.15 ± 0.16 eggs per female per day. Mean daily oviposition did not improve for predators from source EU1 when they were reevaluated 30 days after arrival.

Survival for North American predators ranged between 22.5 and 48.9 percent over the five day test period. Microsporidia-infected predators (EU1) lived longer than uninfected predators from the North American source. Survival for diseased predators ranged from 63.6 to 72.2 percent.

Europe When tested 10 days after arrival, uninfected predators from source EU2 and microsporidia-infected predators (EU1) produced 2.01 ± 0.25 and 1.58 ± 0.17 eggs per female per day, respectively (Table 6.4). There was, however, no significant difference between mean daily oviposition rates of predators from these two sources over the five day test period ($P>0.05$). When performance was reevaluated 30 days after arrival, uninfected predators produced significantly fewer eggs than microsporidia-infected mites from source EU1 during the five day test period ($P<0.001$). Mean oviposition rates of uninfected (EU2) and microsporidia-infected predators (EU1) were 1.15 ± 0.20 and 2.53 ± 0.18 eggs per female per day, respectively.

The survivability of uninfected predators (EU2) ranged from 39.0 to 53.3 percent over five days, whereas the survivability of microsporidia-infected predators (EU1) ranged from 46.5 to 72.3 percent over the same test periods. Although laboratory colonies of predators from sources EU2 and NA were established, survivability remained lower for both these sources than for microsporidia-infected *P. persimilis* from a previously established colony from source EU1.

Sex ratio Uninfected *P. persimilis* females from source EU2 produced significantly more female progeny than microsporidia-infected females from source EU1 ($P<0.005$). The calculated sex ratio for uninfected *P. persimilis* was 2.03:1 (63♀:31♂) and 0.84:1 (43♀:51♂) for females infected with *M. phytoseiuli*.

Lifetime performance

Each female mated with a single male during 24 hr of confinement. Eggs were often produced within the first 24 hr of mating, and in some cases, eggs were present when mating was in progress. Eggs deposited prior to the current mating event indicated that these females had mated more than once with each male. None of the females died before they were mated.

Lifetime oviposition The lifetime mean oviposition rate for uninfected predators (EU2) was significantly higher than the mean oviposition rate of microsporidia-infected predators (EU1) ($P<0.001$) (Table 6.5). Uninfected predators (n=33) produced 3.49±0.14 eggs per female per day, while microsporidia-infected predators (n=36) produced 2.79±.013 eggs per female per day. Uninfected *P. persimilis* females produced a total of 1908 eggs (n=33), while predators infected with *M. phytoseiuli* produced 1269 eggs (n=36).

Mean lifetime oviposition of uninfected and microsporidia-infected predators was 57.8 and 35.3 eggs per female, respectively. Postoviposition survival periods for uninfected predators ranged from 0 to 18 days (average of 2.63 days). Postoviposition survival was shorter for microsporidia-infected predators, ranging from 0 to 13 days (average of 1.28 days).

Age-specific oviposition rates Age-specific oviposition rates, defined as the mean number of eggs oviposited per surviving female, were calculated for both uninfected predators and those infected with *M. phytoseiuli* (Figure 6.1A). Daily oviposition for uninfected predators reached peak rates of 4.39-4.55 eggs per female per day three to five days following mating (10 to 12 days of development) and continued to exceed 3 eggs per female per day until 18 days following mating (25 days of development). Egg production declined rapidly thereafter and eggs were no longer produced 25 days after mating (31 days of development). Peak oviposition rates for microsporidia-infected *P. persimilis* females (3.7 to 4 eggs per female per day) also occurred on days 3 to 5 following mating. Oviposition then decreased more rapidly than among uninfected females. Infected females continued to produce about 3 eggs per female per day (range: 2.8 to 3.8) until 10 days following mating (17 days of development) and declined rapidly thereafter.

Longevity Uninfected predators (EU2) lived significantly longer than microsporidia-infected predators ($P<0.02$). Mean longevity for uninfected predators was 24.58 ± 1.37 days, whereas microsporidia-infected predators lived an average of 19.69 ± 0.84 days (Table 6.5).

Age-specific survival rates Age-specific survival rates, defined as the number of individual predators surviving at each age (observed at 24 hr intervals), were calculated for both uninfected and microsporidia-infected predators (Figure 6.1B). Uninfected females began to die on the seventh day following mating, and the number of live predators decreased gradually until the last female died 33 days following mating (40 days of development). Death of microsporidia-infected predators began on the fourth day following mating, and the last infected female died 28 days after mating had occurred (35 days of development).

Discussion

Performance of Phytoseiulus persimilis Previous studies of *P. persimilis* life history and performance have been conducted under a range of environmental regimes. Differences in experimental design and methodology make comparisons between previous studies and this study difficult. For example, mean prey consumption may vary for *P. persimilis* when different prey are provided; *Tetranychus pacificus* McGregor, *T. cinnabarinus* Boisduval, *T. kanzawai* Kishida and *T. urticae* have all been used as food sources in previous studies (Table 6.6). To further complicate performance comparisons, prey consumption has been reported as either mean eggs or protonymphs consumed per female per day (Laing, 1968; Takafuji and Chant, 1976; Gilstrap and Friese, 1985).

Early life history studies report mean daily oviposition rates of *P. persimilis* between 2.4 and 3.72 eggs per female per day when calculated over the lifetime of ovipositional females. In this study, mean lifetime oviposition rates for both uninfected (EU2) and microsporidia-infected (EU1) *P. persimilis* fall within the range reported in earlier studies. Because predators used in earlier studies were not examined for pathogens, and microsporidia-infected predators in this study performed as well as those in previous studies, it is difficult to determine if predators in past studies were healthy. Results also suggest that predator health

cannot be determined solely on lifetime oviposition values.

Although short-term oviposition rates above 2 eggs per female per day were attainable by *P. persimilis* in this study, mean oviposition rates for uninfected predators (sources EU1, EU2 and NA) ranged from 1.06 to 2.63 eggs per female per day over a five day test period (see Tables 6.1, 6.3 and 6.4). In some cases, low oviposition rates may have been caused by undetected pathogens or by differences in *P. persimilis* strains.

Current quality control guidelines set minimal mean daily oviposition rates of *P. persimilis* at 2 eggs per female per day, with a five day survival rate of 80 percent of egg-laying females (van Lenteren, 1994). Fecundity and longevity tests are to be performed on a seasonal basis under standard conditions (16L:8D; 22-25°C; 70±5% RH) and carried out by the producer prior to shipment. Proposed minimal performance requirements are lower than those reported in earlier life history and performance studies, where lifetime data was used to calculate mean daily oviposition rates (Table 6.6). Because predator health in previous studies was not verified, it remains uncertain if proposed quality control standards are too low, or if *P. persimilis* from commercial sources are capable of meeting these standards. Although the environmental conditions and methodology employed in this study differed slightly from those of proposed quality control tests, methods may be similar enough to allow useful comparisons.

Mean daily oviposition Microsporidia-infected *P. persimilis* females from source EU1 produced significantly fewer eggs over the six day test period than did uninfected females from the same source. When mean oviposition was calculated over five days, uninfected females produced between 2.53 and 2.63 eggs per female per day during two separate studies and exceeded the recommended mean daily oviposition rate of 2 eggs per female per day (van Lenteren, 1994). Predators infected with *M. phytoseiuli* produced 1.61 to 1.72 eggs per female per day. These results suggest that microsporidia-infected predators are not of sufficient quality to be shipped for commercial use. Infected female predators, unable to produce as many eggs as uninfected females, may contribute toward a decline in the predator population, adversely affect establishment of *P. persimilis*

in mass-rearings and ultimately contribute to a reduced capacity for pest control in commercial greenhouses.

Short-term performance of P. persimilis from two commercial sources

Predators obtained from commercial insectaries of North America (NA) and Europe (EU2) performed poorly and mean daily oviposition on Day 5 of testing was well below the recommended rates of 2 eggs per female per day. Mean daily oviposition exceeded recommended rates only when predators from source EU2 were tested 10 days after arrival. At that time, they produced 2.01±0.25 eggs per female per day, slightly more than the minimal mean daily oviposition rate in recommended quality control guidelines (van Lenteren, 1994). In this case, mean daily oviposition of uninfected mites was not significantly different from rates of oviposition among microsporidia-infected mites of an established colony.

When reared under conditions similar to those of this study (25°C; 14L:10D), *P. persimilis* were observed to develop from egg to adult within six days, with adult females surviving on average for 27 days (Takafuji and Chant, 1976). Therefore, most females used for fecundity tests at 30 days following arrival were likely progeny of those originally received. Shipping stresses may have influenced performance results of those predators evaluated at 10 days after arrival. Those tested 30 days after arrival, however, should have become adjusted to rearing conditions used in this study. Based on performance data, results suggest that *P. persimilis* shipped from commercial sources NA and EU2, although not infected with microsporidia, would not pass recommended performance tests.

Poor performance of predators from sources EU2 and NA could be the result of excessive inbreeding. The length of time that predators from these sources were reared without the input of new genetic stock is not known. Genetic inbreeding, however, may not be the sole cause of poor performance. The performance of both uninfected and microsporidia-infected predators from source EU1 often surpassed that of predators from sources EU2 and NA, despite being reared continuously without the introduction of additional genetic stock since December 1993 (see results of short-term performance tests, Tables 6.1, 6.3 and 6.4).

Differences in predator strains may be another possible explanation for variation in performance observed among uninfected predators from sources EU1, EU2 and NA. Galazzi and Nicoli (1994) report significant differences in longevity, oviposition period and total number of eggs produced by three strains of mass-reared and field-collected *P. persimilis* in Europe. The variation in performance among *P. persimilis* strains may be attributed to variations among rearing environments or other factors, but these remain to be studied. Predators are also known to require a period of time to adjust to new prey densities. Eveleigh and Chant (1981) reported that numerical response in *P. persimilis* may be either enhanced or reduced depending on food available. At high prey densities, oviposition of *P. persimilis* remains high, whereas oviposition decreases rapidly when prey densities are low. In this study, predator colonies were fed regularly (twice each week) and leaf discs were excised only from leaves with high spider mite densities. Although standardised as much as possible, fluctuations in prey density could be responsible for some variation in mean oviposition rates.

Although microsporidia were not detected in predators from sources NA and EU2, infection with other pathogens, including virus and rickettsia, were not evaluated. Reduced fecundity of predators from sources NA and EU2 may, therefore, be due to infection with undetected microorganisms. Although the mean oviposition rates of predators from source EU2 did not improve when predators were reevaluated 30 days after arrival, oviposition rates had improved by the time these predators were used to evaluate lifetime performance 12 months after arrival. It is possible that predators exposed to stress during time of shipment require a longer period to overcome stress and establish themselves as a colony. This may have implications for predator release in greenhouses where growers expect timely control.

In two of four studies, mean daily oviposition of infected predators (EU1) exceeded minimal mean oviposition rates of 2 eggs per female per day as outlined in quality control guidelines. Although all females were randomly selected and variation is expected within a population, these results raise the question of whether recommended standards alone would distinguish between infected and

uninfected populations. The ability of infected *P. persimilis* to perform within acceptable standards on occasion reinforces the necessity to continually screen biological control agents for microsporidia and other pathogens.

Performance of *P. persimilis* was assessed for predators where all individuals in a sample were either infected or uninfected with microsporidia. Random sampling of populations with moderate infection rates is more likely the case in commercial settings and performance, including fecundity and survivorship, may remain relatively high in samples where not all individuals are infected. It is more likely that microsporidia will remain undetected under such circumstances and only be discovered when poor performance is noted due to high disease prevalence.

Short-term survival Current quality control guidelines recommend that 80 percent of all females used to evaluate fecundity live for five days of testing (van Lenteren, 1994). In this study, predator survival was highly variable and ranged from 22.5 to 91.2 percent for uninfected predators and 46.5 to 72.7 percent for microsporidia-infected predators when measured over a five day period. Survivorship of uninfected and infected *P. persimilis* from all sources was an unreliable means of confirming the quality of predators, even when considered in association with mean daily oviposition rates.

Prey consumption Infected *P. persimilis* from source EU1 consumed significantly fewer prey than uninfected predators from the same source. Mean prey consumption rates for uninfected ovipositional females were 18.74 and 17.85 deuteronymph *T. urticae* per day over five and six days of testing, respectively. Microsporidia-infected females consumed 13.39 and 13.02 prey protonymphs over the same periods. Results for uninfected predators are consistent with those of Takafuji and Chant (1976) who report 17.6 protonymph *T. pacificus* consumed per female per day.

Prey consumption studies are not included in current quality control guidelines as a means to assess predator quality. Although evaluation of *P. persimilis* for mean prey consumption proved time-consuming and was not the most practical means to evaluate performance, prey consumption data may assist

in the determination of predator quality. *Phytoseiulus persimilis* females in this study did not consume all prey confined within observation dishes and, therefore, prey consumption by *P. persimilis* was not affected by temporary starvation. As prey were consumed over each 24 hr observation period, however, decreases in prey density could contribute to an increase in searching time by *P. persimilis* and affect overall mean prey consumption. Low prey densities would have a greater effect on prey searching by healthy mites since they consume more prey over a shorter period.

Lifetime oviposition Mean lifetime oviposition rates for *P. persimilis* were higher than oviposition rates calculated over five and six days during short-term performance tests. Mean lifetime oviposition rates observed for uninfected and microsporidia-infected predators (3.49 and 2.79 eggs per female per day, respectively) are within the range reported for *P. persimilis* in previous studies (Table 6.6). Data of lifetime oviposition rates would be insufficient to indicate predator quality without examination of individual predators for microsporidian spores. Likewise, presence of disease cannot be confirmed from low oviposition rates during short-term studies without confirmation of pathogens by microscopic examination of individuals.

Repeat matings were observed for some *P. persimilis* females when confined with a single male for a 24 hour mating period. Although repeat matings are a known part of normal reproductive behaviour (Laing, 1968), one copulation event is sufficient for egg production and oviposition (Schulten *et al.*, 1978). Mating events for *P. persimilis* are reported to last as long as 130 min and, although oviposition occurs in females mated for 30 min or longer, total egg production decreases when copulation is of shorter duration. Because disturbed mating events have resulted in reduced oviposition periods and lowered daily oviposition rates (Amano and Chant, 1978), mating pairs observed *in copula* in my study were left undisturbed. Males were removed only after uncoupling occurred.

Age-specific oviposition and survival rates The age-specific oviposition curve (Figure 6.1A) observed for uninfected predators resembles those reported for *P. persimilis* reared at 25°C by McClanahan (1968) and Takafuji and Chant (1976).

In their studies, peak oviposition was observed three to five days after mating, followed by a gradual decline in the number of eggs produced until the end of the oviposition period. In this study, peak oviposition for uninfected predators was also observed three to five days after mating. Uninfected predators produced in excess of 3 eggs per female per day until 18 days following mating, or approximately half of their lifetime.

Peak oviposition for microsporidia-infected predators occurred three to five days after mating and mean oviposition remained above 3 eggs per female per day during the first 10 days following mating, or about one-third of their lifetime. Oviposition curves for microsporidia-infected predators showed a sharp decline in the number of eggs produced after peak oviposition occurred. Microsporidia-infected predators survived for prolonged periods while producing few or no eggs and infected predators therefore may contribute little toward an increase in the predator population as a whole.

Both uninfected (EU2) and infected predators continued to feed on spider mites during their postovipositional periods. Because uninfected female predators had longer postovipositional periods and lived significantly longer than did microsporidia-infected predators, uninfected female *P. persimilis* are likely to consume more prey and further contribute toward the reduction of spider mite populations.

Sex ratio The sex ratio for uninfected *P. persimilis* from source EU2 was calculated at 2.03:1 (63♀:31♂). This is considerably lower than the sex ratios, ranging from 3:1 to 4:1 (♀:♂), reported in previous studies (Kennett and Caltagirone, 1968; Laing, 1968; Takafuji and Chant, 1976; Scopes, 1985). The variations in sex ratio reported in earlier studies may be attributed to variations in experimental conditions, or to genetic variability among the different strains of *P. persimilis* or to some other factor. A reduction in the proportion of females in the population could reduce the intrinsic rate of increase.

Microsporidia-infected females from source (EU1) produced significantly fewer female progeny than uninfected females (EU2). Their sex ratio was calculated at 0.84:1 (43♀:51♂). Kennett and Caltagirone (1968) report sex ratio

distortion of 1.1:1.0 in progeny arising from starved *P. persimilis* females collected from strawberry plants with very low predator densities. All predators in my study were fed regularly and prey were observed in abundance within rearing cages upon sampling. It is therefore unlikely that sex ratio distortions were starvation-induced. Sex ratio distortion, as noted for microsporidia-infected *P. persimilis*, may have pronounced effects on reproductive potential and affect the intrinsic rate of increase as defined by Laing (1968). Sex ratio distortion, in addition to low fecundity and prey consumption rates of microsporidia-infected mites, may further contribute toward difficulties in the establishment of predators and in the control of pest mite populations within commercial greenhouses.

Although *P. persimilis* infected with microsporidia lack visible external signs (Bjørnson *et al.*, 1996), infected female predators appeared smaller than uninfected females. Size differences were not quantified, but uninfected female progeny are larger than uninfected males and sex can be determined on the fifth day of development. In contrast, microsporidia-infected males and females are similar in size and sex determination is less certain until the sixth day of development. Others have observed that adult parasitoids infected with microsporidia are smaller than their uninfected counterparts (Laigo and Tamashiro, 1967; Saleh, *et al.*, 1995) and less robust at emergence (Allen, 1954). Microsporidia have also been thought responsible for causing malformations in adult parasitoids that contribute to problems with dispersal (Lipa and Steinhaus, 1959). Malformations, however, were not observed in infected *P. persimilis*. While microsporidia may have profound effects on searching behaviour of *P. persimilis*, this remains to be studied.

More than 600 species of microsporidia have been described from insect hosts. Associated diseases range from chronic to highly virulent (Bylen, 1994). This study has addressed the effects of one microsporidium, *Microsporidium phytoseiuli*, on the performance of *Phytoseiulus persimilis*. Three microsporidian species have been observed in *P. persimilis* obtained from three commercial sources including North America, Israel, and Europe (Bjørnson *et al.*, 1996). Other microsporidian species, alone or in combination, may have more or less pronounced effects on host performance, but these remain to be studied.

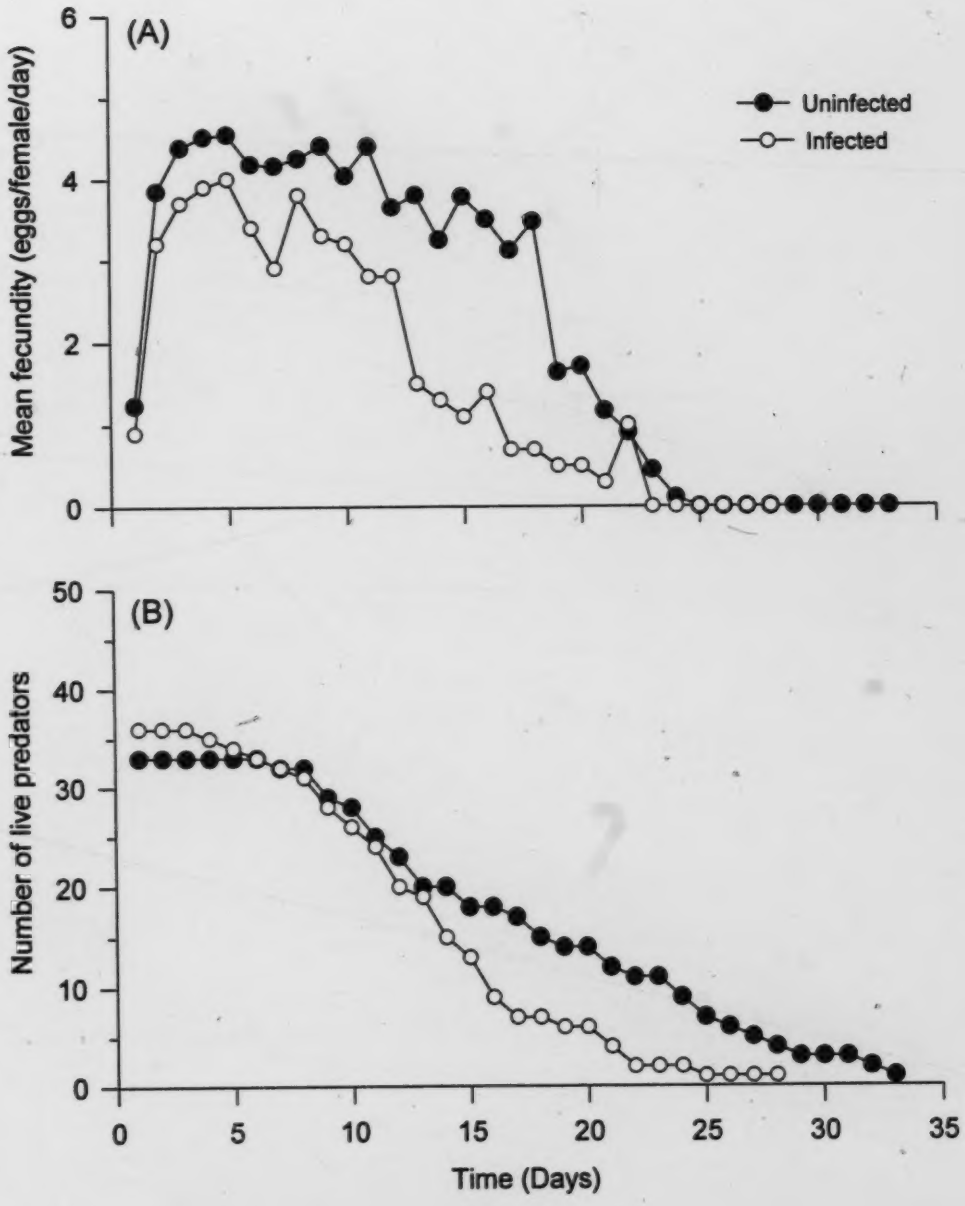

Figure 6.1. Age-specific oviposition (A) and age-specific survival curves (B) of uninfected *Phytoseiulus persimilis* females and of those infected with *Microsporidium phytoseiuli*. All females were provided excess *Tetranychus urticae* prey. Day 0 represents the seventh day of development (when mating occurred); Day 1 represents the first day that eggs were counted following mating.

Table 6.1

Mean Daily Oviposition Rates and Percent Survival of *Phytoseiulus persimilis* from a Commercial Insectary of Europe (EU1)

	Mean Daily Oviposition Rates (eggs/female/day)									Percent Survival		
	5 Days				6 Days							
	Min	Max	Mean±S.E.	P	Min	Max	Mean±S.E.	P	n_e	Day 5	Day 6	n
Uninfected	0.00	5.00	2.63±0.24a	<0.005	0.00	4.83	2.60±0.23a	<0.002	706	58.7	47.8	46
Infected	0.00	4.40	1.72±0.18b		0.00	4.33	1.66±0.18b		521	60.8	43.1	51
Uninfected†	0.67	4.20	2.53±0.13a	<0.002	0.67	4.17	2.38±0.13a	<0.001	523	91.2	88.2	34
Infected†	0.00	3.60	1.61±0.20b		0.00	3.50	1.50±0.19b		352	72.7	63.6	33

†, fecundity recorded during prey consumption studies; n_e, total number of eggs produced. Means within columns followed by different letters are significantly different.

Table 6.2
Prey Consumption Rates† of *Phytoseiulus persimilis* from a Commercial Insectary of Europe (EU1)

	5 Days				6 Days					
	Min	Max	Mean±S.E.	P	Min	Max	Mean±S.E.	P	n_p	n
Uninfected	6.40	29.67	18.74±1.01a	<0.002	6.67	29.67	17.85±0.96a	<0.005	3670	34
Infected	1.50	30.20	13.39±1.20b		1.50	30.33	13.02±1.15b		2812	33

†, mean deuteronymph *T. urticae* eaten per female per day; n_p, total number of prey consumed. Means within columns followed by different letters are significantly different.

Table 6.3
Mean Oviposition Rates and Percent Survival of *Phytoseiulus persimilis* Obtained from a Commercial Source in North America (NA)

	Mean Oviposition Rate (eggs/female/day)								Percent Survival		
	5 Days				6 Days						
Source	Min	Max	Mean±S.E.	P	Min	Max	Mean±S.E.	P	5 Day	6 Day	n
EU1(U)†	0.00	5.00	2.63±0.24		0.00	4.83	2.60±0.23		58.7	47.8	46
NA(U) D10	0.00	5.00	1.06±0.19a	<0.001	0.00	4.83	1.06±0.19a	<0.001	22.5	15.0	40
EU1(I)	0.00	3.80	2.15±0.16b		0.00	3.67	2.07±0.16b		72.2	58.3	36
NA(U) D30	0.00	4.20	1.06±0.17a	<0.005	0.00	4.00	1.00±0.16a	<0.005	48.9	33.3	45
EU1(I)	0.00	4.20	1.84±0.21b		0.00	3.83	1.73±0.19b		63.6	52.3	44

U, no microsporidia detected; I, microsporidia detected. Means within columns followed by different letters are significantly different. D10, tested 10 days after arrival; D30, tested 30 days after arrival. †Values established during previous performance studies (see Table 6.1); Colony performance used for comparative purposes only.

Table 6.4
Mean Oviposition Rates and Percent Survival of *Phytoseiulus persimilis* Obtained from a Commercial Source in Europe (EU2)

	Mean Oviposition Rate (eggs/female/day)								Percent Survival		
	5 Days				6 Days						
Source	Min	Max	Mean±S.E.	P	Min	Max	Mean±S.E.	P	5 Day	6 Day	n
EU1(U)†	0.00	5.00	2.63±0.24		0.00	4.83	2.60±0.23		58.7	47.8	46
EU2(U) D10	0.00	5.00	2.01±0.25a	>0.05	0.00	5.00	1.96±0.24a	>0.05	39.0	24.4	41
EU1(I)	0.00	4.20	1.58±0.17a		0.00	4.17	1.51±0.16a		46.5	39.5	43
EU2(U) D30	0.00	5.00	1.15±0.20a	<0.001	0.00	5.17	1.12±0.20a	<0.001	53.3	31.1	45
EU1(I)	0.00	4.40	2.53±0.18b		0.00	4.17	2.41±0.18b		72.3	70.0	47

U, no microsporidia detected; I, microsporidia detected. Means within columns followed by different letters are significantly different. D10, tested 10 days after arrival; D30, tested 30 days after arrival. †Values established during previous performance studies (see Table 6.1); Colony performance used for comparative purposes only.

Table 6.5
Lifetime Mean Oviposition Rates and Longevity of *Phytoseiulus persimilis* from Two Commercial Sources

	Mean Oviposition Rate (eggs/female/day)					Longevity (Days)				
	Min	Max	Mean±S.E.	P	n_e	Min	Max	Mean±S.E.	P	n
EU2 (U)	0.48	4.36	3.49±0.14a	<0.001	1908	13.0	40.0	24.58±1.37a	<0.02	33
EU1 (I)	0.00	3.64	2.79±.013b		1269	10.0	28.0	19.69±0.84b		36

U, no microsporidia detected; I, microsporidia detected; n_e, total number of eggs produced. Means within columns followed by different letters are significantly different.

Table 6.6
Mean Oviposition Rates, Longevity and Prey Consumption Rates Reported for Ovipositional *Phytoseiulus persimilis* Females

Author(s)	Rearing Conditions	Mean Lifetime Oviposition (eggs/♀/day)	Lifetime Oviposition (total eggs/♀)	Adult Female Longevity (days)	Prey Consumed (/♀/day)	Sex Ratio (♀:♂)	Prey
Kennett and Caltagirone (1968)	72-80°C	3.2-3.7	49.3-64.8[1]			3.8:1	*Tetranychus urticae* Koch
Laing (1968)	58.5-83°F 15.5L:8.5D 65-95% RH	2.4±0.3	53.5	29.6	7.3±1.2 eggs[2] 14.3±1.9 eggs[3] 3.9±1.5 eggs[4]	4.1:1	*T. urticae*
McClanahan (1968)	20±0.5°C 26±0.5°C 90% RH; no light		43.8±23.3 (20°C) 53.5±20.7 (26°C)				*T. urticae*
Ashihara *et al.* (1976)	22-25°C	3.5			>20 eggs		*T. kanzawai* Kishida
Takafuji and Chant (1976)	25±1°C 75-90% RH 14L:10D	3.7±0.1	79.5±4.6	36.4±1.7	9.1±0.9 protonymphs[2] 17.6±1.5 protonymphs[3] 3.8±1.2 protonymphs[4]	4.6:1[1]	*T. pacificus* McGregor
Amano and Chant (1977)	23±1°C 55±10% RH 12-15hL/24 hr	2.9±0.2	66.3±15.3	47.5±24.9			*T. pacificus*
Gilstrap and Friese (1985)	26.1±2.8°C 50±20% RH 14L:10D				25 eggs[1]		*T. cinnabarinus* Boisduval

Table 6.6 (continued)
Mean Oviposition Rates, Longevity and Prey Consumption Rates Reported for Ovipositional *Phytoseiulus persimilis* Females

Author(s)	Rearing Conditions	Mean Lifetime Oviposition (eggs/♀/day)	Lifetime Oviposition (total eggs/♀)	Adult Female Longevity (days)	Prey Consumed (/♀/day)	Sex Ratio (♀:♂)	Prey
Galazzi and Nicoli (1994)	25°C 80±5% 16L:8D	(SI) 3.3±1.0	48.0±24.8	14.9±7.1	23.5 eggs[1]	3.95:1[1]	*T. urticae*
		(NE) 2.2±1.2	22.3±24.0	8.3±6.0	29.6 eggs[1]	1.78:1[1]	
		(CE) 2.7±1.3	25.1±21.7	8.9±5.4	26.9 eggs[1]	1.82:1[1]	
Gillespie and Quiring (1994)	26°C 16L:8D	3.0±0.5	56.0±5.7	20.7±2.7			*T. urticae*
		3.4±0.3[5]	38.1±3.3[5]	11.3±0.6[5]			

[1]estimated from data tables; [2]preoviposition period; [3]oviposition period; [4]postoviposition period; [5] *P. persimilis* fed spider mites on tomato leaves. SI, NE and CE refer to three different *P. persimilis* strains of differing origin, as reported by Galazzi and Nicoli (1994).

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Chapter 7

Disease Prevalence and Modes of Transmission of *Microsporidium phytoseiuli*, Infecting the Predatory Mite, *Phytoseiulus persimilis*

Introduction

Pathogen persistence in a host population is dependent, in part, on its ability to be successfully transmitted (Andreadis, 1987). Pathogen transmission can be defined as horizontal or vertical, depending on the manner in which pathogens are disseminated among individuals within the population. *Horizontal transmission* is defined as transmission of pathogens from one individual to another within or between generations. This includes autoinfection, where a host becomes reinfected with the same pathogen. *Vertical transmission* is defined as the direct transfer of a pathogen from parent to progeny, and often occurs in or on the surface of an egg (Andreadis, 1987; Tanada and Kaya, 1993). Vertical transmission in univoltine insects helps ensure survival between host generations that do not overlap (Canning *et al.*, 1985).

Horizontal transmission in insects occurs when pathogens gain entry to the host through natural body openings (mouth, anus, spiracles/gills) or through the integument. Among protozoa, pathogen invasion through the mouth (*per os*) is the most prevalent form of transmission. It often results from the ingestion of contaminated food or faecal material, consumption of infected cadavers, or cannibalism of infected hosts. Although host invasion through the anus and spiracles has been recorded for entomopathogenic nematodes, it is an uncommon transmission mechanism for most insect pathogens, including protozoa. Direct invasion through the integument is rare among protozoa, although indirect cuticular transmission of microsporidia may occur through contaminated ovipositors of hymenopterous parasitoids (Andreadis, 1987; Brooks, 1993; Tanada and Kaya, 1993).

Vertical transmission is the principal means of transmission for microsporidia that infect mosquito and many lepidopteran hosts (Tanada and Kaya, 1993). Development of microsporidiosis in most mosquito hosts involves vertical transmission between successive mosquito generations and horizontal transmission between mosquitoes and intermediate copepod hosts (Andreadis,

1990; Becnel, 1992; Sweeney *et al.*, 1993; White *et al.*, 1994). Selective transmission in these hosts is thought to be associated with spore dimorphism (Garcia *et al.*, 1993; Hazard and Fukuda, 1974) where heterosporous microsporidia produce more than one spore type, each responsible for a specific type of transmission. For other insects, vertical transmission augments horizontal routes of infection and permits pathogens to persist when host population densities are low (Andreadis, 1987).

Vertical transmission from infected female to progeny is divided into two categories. Transovarial transmission occurs when the pathogen gains direct entry into the egg while within the female host through infection of the ovaries and/or associated reproductive structures. Microsporidian spores or sporoplasms are incorporated into the egg or embryo within an infected female, with subsequent progeny becoming infected during development (Tanada and Kaya, 1993). Transovum transmission occurs when spores contaminate the egg surface and are consumed by larvae during eclosion. A newly-emerged larva may become infected when spores are ingested with yolk or as the larva eats through the egg shell at emergence. In the case of the winter moth, *Operophtera brumata* L., microsporidian spores accumulate within the yolk but do not infect the tissues of developing embryos. In this case, the egg provides a site for the protection of spores from the environment although the infection route is considered oral, in the strict sense (Canning *et al.*, 1985). Transovarial transmission has been reported more frequently than has transovum transmission. Some microsporidia are vertically transmitted from adult males to their progeny by venereal transfer to the female parent during mating and the subsequent transfer to the egg (Thomson, 1958; Kellen and Lindegren, 1971; Toguebaye and Marchand, 1984). However, transmission between sexes is rare (Tanada and Kaya, 1993).

Although microsporidian transmission has been investigated for several insect pests, primarily those of agricultural or veterinary importance, few studies have examined transmission in beneficial arthropods. In *Muscidifurax raptor*, a pteromalid parasitoid used for control of muscoid flies, the microsporidium *Nosema mucidifuracis* produces diplokaryotic spores with three distinct

morphologies, each thought responsible for a specific mode of transmission. One spore type is believed to be responsible for autoinfection, a second for horizontal transmission, and the third, found within the eggs of *M. raptor*, for either initiation and spread of infection at eclosion or for horizontal infection to a new host when eggs are cannibalised (Becnel and Geden, 1994). Transmission has also been investigated for two primary and secondary hymenopterous parasitoids of *Heliothis* species. The primary parasitoid *Campoletis sonorensis* is capable of becoming infected with *Nosema heliothidis*, a microsporidium that infects the corn earworm, *Heliothis zea*. This microsporidium is transovarially transmitted by infected parasitoid females (Brooks, and Cranford, 1972). The hyperparasitoids *Catolaccus aeneoviridis* (Pteromalidae) and *Spilochalcis side* (Chalcididae) do not become infected with *N. heliothidis*. However, these can become infected with a second microsporidium, *Nosema campoletidis* after they develop within infected *C. sonorensis*. Although transovarial transmission of *N. campoletidis* was reported, the microsporidium had no detrimental effects on these hyperparasitoids (McNeil and Brooks, 1974).

Transmission and dispersal, spore longevity, host range and infectivity, and environmental factors all contribute to pathogen infection rates. All are important factors in the persistence of microsporidia in any environment (Maddox, 1973). Therefore, management and control of microsporidia within commercial insectaries may be difficult to achieve without information regarding the modes of transmission and dispersal of these pathogens. The aim of this study was to determine disease prevalence, defined as the number of hosts afflicted with a disease at a given point in time (Fuxa and Tanada, 1987), of *Microsporidium phytoseiuli* in two colonies of *P. persimilis*. Vertical and horizontal modes of transmission of *M. phytoseiuli* were investigated and parental contribution toward infection (maternal and paternal transmission) is reported.

Materials and Methods

Mite rearing *Phytoseiulus persimilis* used in this study were obtained from a commercial source in Europe (EU1) in December 1993. Predators were reared under quarantine conditions at the Alberta Research Council (Vegreville, AB).

Predators from the initial shipment (n=1000) were placed on *Tetranychus urticae*-infested bean plants (*Phaseolus vulgaris*) within two separate cages. Each cage contained two infested bean plants. Predators were fed twice each week by replacing one of the caged plants with a fresh, spider mite-infested plant. Used pots and soil were discarded. To prevent movement between predator colonies, each cage was suspended over a moat of soapy water. Cages were isolated on separate benches in a greenhouse operating under controlled environmental conditions (16L:8D; 25°C:20°C). All predators used for determining disease prevalence were sampled from one cage unless stated otherwise. Despite attempts to maintain disease-free predator colonies, all uninfected *P. persimilis* from source EU1 eventually became infected with *M. phytoseiuli* (see Chapter 6). Predators obtained from a second European source (EU2) were used in horizontal transmission studies and to determine parental contribution toward infection. These were reared under controlled environmental conditions in growth chambers at the University of Alberta (16L:8D; 25°C:20°C).

Observation dishes Observation dishes used to study parasite transmission were described in Chapter 6 (see also Appendix 4). Observation dishes were placed in larger plastic containers and kept in an incubator (16L:8D; 25°C:20°C).

Disease prevalence Each week, 96 immature and adult predatory mites (source EU1) were randomly selected from Cage 1. Samples consisting of both living and dead predators were taken over 57 weeks. Over a shorter period, corresponding to weeks 51 to 57 (of EU1) and continuing to week 68, monthly random samples (n=96) of *P. persimilis* were collected from Cage 2 and examined. Predators were smeared on glass slides, fixed in methanol, and stained with 15% buffered Giemsa. Disease prevalence was calculated as percent infected with microsporidian spores. Samples of 40 prey mites (*T. urticae*) were removed biweekly during this period and examined for microsporidian spores.

Light and scanning electron microscopic (SEM) observations Faecal pellets from infected predators were removed from bean foliage, smeared in distilled water, fixed in methanol, stained with 15% buffered Giemsa, and examined for microsporidian spores using a light microscope. For examination by SEM, bean

leaves previously exposed to microsporidia-infected mites were collected and air-dried. The lower epidermis of four leaves were gold-coated and examined for microsporidian spores with a Jeol JSM.6301FXV field emission scanning electron microscope (accelerating voltage, 5 kV).

Transmission studies

Vertical transmission of M. phytoseiuli Gravid *P. persimilis* females were randomly selected and removed from leaves with a fine bristle paint brush. Females were isolated on excised leaf discs in observation dishes and observed once every 24 hr for seven days. Leaf discs were replaced once during the seven day study. One egg laid within the first 24 hours of the observational period was taken from each female and smeared and examined for microsporidian spores. Subsequent eggs were permitted to hatch in Millipore dishes that had been partitioned into seven sections. High ambient relative humidity was maintained with small pieces of moistened filter paper placed within dishes. Percent hatch and percent infection were recorded.

Although microsporidian spores were observed in eggs laid by infected females when examined by both light and transmission electron microscopy (Bjørnson *et al.*, 1996), tests were undertaken to determine whether the spores within *P. persimilis* eggs were capable of causing infection. In one study, 10 eggs from an infected female were surface sterilised in 3% bleach solution (3 min), followed by 1% sodium thiosulphate (1 min), then rinsed with sterile water. Eggs were isolated, permitted to hatch and to develop into adults which were then smeared and examined for microsporidian spores. In another study, a single infected male and female were mated and the infection status of all subsequent progeny were recorded over five generations. The original female parent was permitted to produce eggs for seven days. Male and female progeny were mated and their progeny were examined for microsporidian spores as newly-hatched larvae or after they had developed into adults.

Horizontal transmission of M. phytoseiuli Four experiments were designed to examine horizontal transmission. In three of these, uninfected adult predators were exposed to either contaminated foliage or to infected adult predators. In the

fourth experiment, uninfected immature predators were allowed to develop in proximity to infected adult and immature predators.

In the first experiment, uninfected females were confined to leaf surfaces previously exposed to microsporidia-infected predators. Adult females from a microsporidia-infected colony (EU1) were transferred to spider mite-infested leaf discs in observation dishes where they remained for 48 hr. These females were then removed and examined to confirm infection status. Uninfected females were then transferred to these leaf discs where they remained for seven days. After this period, predators were smeared on glass slides, stained in 15% buffered Giemsa, and examined for infection. In a second experiment, uninfected females were exposed to leaf surfaces directly contaminated with microsporidian spores obtained either as crushed, infected eggs or as a suspension of spores within distilled water. Predators were examined after seven days of exposure. In the third experiment, uninfected adult females were marked with acrylic paint and placed within arenas containing live, infected female predators. Uninfected females were exposed to microsporidia-infected predators for 48 hr and examined for microsporidian spores seven days after exposure.

In the fourth experiment, individual uninfected immature mites were exposed to infected adult *P. persimilis* throughout their development. Uninfected gravid *P. persimilis* females were placed in observation dishes and all eggs removed after 24 hr. Each egg was isolated on a *T. urticae*-infested leaf disc in an observation dish. Parent females were then examined to confirm infection status. Eggs collected from uninfected females were permitted to hatch and develop further for 24 to 48 hr, depending on the developmental rate of newly-hatched larvae. Upon development into nymphs, five infected *P. persimilis* adult females were placed within each dish. Predators were observed daily and all eggs produced by the infected adult females were removed. Infected adults remained in the same observation dish as the developing immature for two to seven days and were removed only when the developing immature reached a size comparable to that of infected adults. Upon removal, adult females were smeared and examined to confirm infection status. All eggs produced by infected adults on the

day of their removal were left to hatch. All resulting immatures remained on the leaf disc with the uninfected predator which had now developed into an adult. This procedure ensured that uninfected immatures were in contact with infected mites for a minimum of five days during development. Predators originating from uninfected females were removed after 14 days, or upon death, and examined for microsporidian spores.

Parental contribution toward infection Eggs collected from both uninfected and infected *P. persimilis* females were isolated on leaf discs within observation dishes. Adult female predators were then examined for microsporidian spores to determine infection status. Eggs were observed daily. Progeny were sexed and, on the sixth day following egg collection and isolation, a single virgin male was placed in each dish along with a single virgin female. Males remained with females for 24 hr so that mating would occur. Males were then removed and examined to confirm infection status. Predators were mated in four mating combinations as follows: uninfected female X uninfected male (n=17), uninfected female X infected male (n=16), infected female X uninfected male (n=18), and infected female X infected male (n=18). Eggs were collected from all females of each mating combination, stained with Giemsa and examined for microsporidian spores to determine the parental contribution toward infection of the progeny. Significance among means was determined using a one-way ANOVA and Bonferroni multiple comparison test.

Results

General observations Prey exuviae, unhatched eggs, and remnants of hatched eggs were observed on the undersurface of bean leaves when examined by SEM. Microsporidian spores were not observed on leaf surfaces nor in predator faeces, which were readily identified by SEM as intact aggregates comprised of numerous dumbbell-shaped crystals. However, few to many microsporidian spores were observed in smeared faecal preparations examined by light microscopy.

Disease prevalence Disease prevalence of predators from Cage 1 remained relatively low and fluctuated between 0 and 15 percent over 57 weeks of observation (Figure 7.1A). Disease prevalence of predators from Cage 2

increased from 12 to 96 percent over two months and then to 100 percent (Figure 7.1B). Disease prevalence of predators in Cage 1 increased to 100 percent approximately two months after weekly sampling had ceased for this colony (data not shown). Periodic sampling has since confirmed 100 percent infection in both of these predator colonies.

Parasite transmission

Vertical transmission Egg hatch was 100 percent for eggs collected from both infected and uninfected predators (n=165). Vertical transmission of *M. phytoseiuli* from infected female progeny was 100 percent (n=150). All progeny that hatched from surface sterilised eggs were infected with microsporidian spores. All progeny from microsporidia-infected female predators were infected with microsporidian spores when examined as newly-hatched larvae, immatures or adults. When observed over five generations, all progeny produced by a single mated pair (infected male and female) were infected with microsporidian spores.

Horizontal transmission All attempts to infect adult *P. persimilis* with *M. phytoseiuli* were unsuccessful. Horizontal transmission did not occur when uninfected adult predators were placed on leaf surfaces previously exposed to infected predators, nor when exposed to leaf surfaces to which microsporidian spores were applied. Uninfected adult female predators marked with paint and placed within arenas that contained infected predators often died within a few days but remained uninfected. Horizontal transmission of *M. phytoseiuli* did occur when immature *P. persimilis* were permitted to develop in contact with infected immature and adult predators. The horizontal transmission rate was low as only six of 42 predators (14.3 percent) were infected. One of the six predators to become infected died five days after exposure. Examination of this individual after death showed it to be infected with microsporidia. The remaining five predators that became infected lived an average of 12 days (range: 10 to 13 days) after initial exposure to infected individuals.

Parental contribution toward infection The number of eggs examined from all females of all four mating combinations was as follows: uninfected female X uninfected male (n=444), uninfected female X infected male (n=630), infected

female X uninfected male (n=251), and infected female X infected male (n=270). Microsporidian spores were not observed in any of the eggs examined from uninfected females, regardless of whether they were mated with uninfected or infected males (Table 7.1), whereas all eggs examined from infected females contained microsporidian spores, regardless of the infection status of the males.

A one-way ANOVA showed differences between the mean number of eggs produced by females from the four mating groups ($F=10.518$; $P<0.001$, $df=3,65$; Table 7.1). The mean number of eggs produced per mated pair was highest when uninfected females were mated with uninfected males (Table 7.1). In this case, 63.2 eggs were produced per mated pair. When uninfected females were mated with infected males, 52.1 eggs were produced per mated pair. There was no significant difference ($P>0.05$), however, between the mean number of eggs produced by uninfected females when mated with either uninfected or infected males.

Microsporidia-infected females produced significantly fewer eggs ($P<0.05$) than uninfected females. Infected females produced 31.3 eggs per mated pair when mated with uninfected males, whereas infected females produced 39.2 eggs per mated pair when mated with infected males. There was no significant difference ($P>0.05$) between the mean number of eggs produced by uninfected females when mated with infected males and infected females mated with infected males (Table 7.1).

Discussion

Disease prevalence Microsporidia that cause chronic infections often remain undetected within host populations (Goodwin, 1984). Low disease prevalence and lack of obvious disease signs or symptoms, as in the case of *M. phytoseiuli*, increase the probability that these pathogens will escape notice unless individuals are routinely examined for pathogens. During low disease prevalence, *M. phytoseiuli* was not always detected when *P. persimilis* colonies were examined for microsporidia, despite weekly sample sizes of 96 predators.

Although disease incidence remained low for a considerable period in one colony, both this routinely monitored population and a second colony reached 100

percent disease prevalence. This change in disease status may be attributable to one or more abiotic or biotic factors. Although predator colonies were reared under controlled environmental conditions, slight variations in temperature, light, or humidity may have contributed toward an increase in disease prevalence. Environmental conditions responsible for the increase in disease prevalence of *M. phytoseiuli* were not investigated.

In addition to environmental stresses, several biotic factors could be responsible for an increase of disease prevalence. These include population susceptibility (genetic resistance, age), population characteristics (predator density, cannibalism, behaviour), and pathogen characteristics (virulence, pathogen dispersal). Temporary starvation and overcrowding are often unavoidable in mass-rearings. These place stresses on individuals, presumably making them more susceptible to disease. Although predator colonies were fed twice each week, prey mites occasionally became scarce due to high predator density. Microsporidian spores were not detected in *Tetranychus urticae* when routinely examined by light microscopy. Therefore, it seems unlikely that *T. urticae* contributed toward the observed increase of disease prevalence.

Parasite transmission *Microsporidium phytoseiuli* should remain at relatively low levels within the host population if vertical transmission is the sole means of pathogen dispersal among individuals. Reduced fecundity and longevity of microsporidia-infected predators (see Chapter 6) would put these at a disadvantage in a colony consisting of both infected and uninfected individuals. Low incidence of disease in Cage 1 over 57 weeks supports this hypothesis.

Vertical transmission Vertical transmission is responsible for maintaining the pathogen at low levels within the host population. High vertical transmission rates, as observed for *M. phytoseiuli*, may cause difficulties in controlling this microsporidium within the host population.

It is possible that disease prevalence remains low when predator densities are low. As predator densities increase and age structure within the population changes, a higher proportion of immatures are exposed to microsporidian spores. As a critical level of inoculum is reached, likely above 15 percent disease

prevalence, an increasing proportion of individuals becomes infected through vertical transmission. Remaining uninfected individuals produce eggs that develop into immature predators with an increased chance of infection through horizontal transmission. These have increased chance of exposure as they develop in proximity of infected predators. Disease prevalence may increase when individuals are subjected to temporary stresses, including environmental fluctuations and low prey densities. Although not observed, cannibalism may occur when prey mites become scarce due to high predator densities, and may contribute toward the horizontal transmission of *M. phytoseiuli* in *P. persimilis* colonies. Overcrowding of predator colonies may also increase the probability of horizontal transmission by increasing the likelihood that uninfected predators will come in direct contact with those infected with microsporidia. The transmission of microsporidia from infected adults to immature *P. persimilis* supports this hypothesis.

Horizontal transmission In initial attempts to transmit *M. phytoseiuli*, it was difficult to ensure that uninfected adult predators came in contact with microsporidian spores. Furthermore, some uninfected adults died prior to the end of the seven day test period and likely before horizontal infection could be detected. Assuming that horizontal transmission was successful, it is possible that the death of adult hosts occurred before enough time had lapsed for the pathogen to invade and develop within host tissues. Due to the difficulty of identifying early life cycle stages of *M. phytoseiuli*, microsporidia may have been overlooked in smear preparations examined by light microscopy.

Horizontal transmission was observed only when uninfected immature predators were allowed to develop in proximity to adult and immature predators infected with *M. phytoseiuli*. This may be attributed, in part, to prolonged exposure of uninfected immature predators to those infected with microsporidia. Even so, the horizontal transmission rate was low, about 14.3 percent for those individuals tested.

Successful horizontal transmission of *M. phytoseiuli* may be dependent on the ability of the parasite to successfully invade developing tissues of the immature

host. As the immature predator develops, the microsporidium has the opportunity to proliferate and infect all host tissues. Immature *P. persimilis* may be more susceptible to infection by *M. phytoseiuli* than adult predators. Spores may be infectious only to immature hosts and thereby contribute toward noticeable infection levels only when the proportion of immature predators is high within the population.

Spore viability of some microsporidian species are known to be influenced by environmental factors such as temperature, light exposure, and relative humidity. Spore infectivity and persistence for most species of microsporidia have not been investigated (Maddox, 1973). It is possible that *M. phytoseiuli* spores on exposed leaf surfaces were non-viable and incapable of causing infection.

Horizontal transmission reported for *M. phytoseiuli* in this study may be lower than in commercial mass-rearings. Uninfected *P. persimilis* in commercial insectaries may be subjected to high predator densities on a localised scale. For example, high prey densities on the foliage of individual plants within a rearing facility may attract and sustain many predators, while their progeny contribute toward even higher predator numbers within the surrounding area. When microsporidia-infected hosts are present, an increase in the number of predators within a localised area could translate to an ever-increasing opportunity for these predators to come in direct contact with infected predators during their development. Immature predators may continue to be exposed to infected eggs, immatures, and adult predators throughout their lifetime. This may increase the horizontal transmission of microsporidia, especially if low prey densities encourage cannibalism.

Examination of faecal smears by light microscopy revealed that variable numbers of microsporidian spores are excreted by infected predatory mites. When examined by SEM, however, spores were not observed on lower leaf surfaces previously exposed to infected predators. Neither were spores observed within intact faecal aggregates. In the latter case, faecal pellets seemed to consist primarily of dumbbell-shaped crystals, and these may have concealed any microsporidian spores that were present. Faecal masses on foliage surfaces

appeared as solid aggregates when examined by SEM. It seems unlikely that microsporidian spores would be liberated from dry faecal masses onto leaf surfaces. However, faecal pellets dissolved readily in distilled water and it is possible that faecal masses may collapse or break apart while on leaf surfaces if ambient humidity is high or when water is splashed directly onto foliage. Although spores could be liberated onto leaf surfaces under such conditions, spore viability under these conditions is unknown. Unsuccessful transmission of *M. phytoseiuli* by the direct application of spores to leaf surfaces supports the assumption that horizontal infection is unlikely to occur through casual contact with contaminated surfaces.

Parental contribution toward infection There was no evidence to suggest that microsporidia were transmitted from infected males to uninfected females. Maternal transmission, however, was 100 percent. Uninfected females always produced uninfected eggs, regardless of the infection status of the male, and infected females always produced eggs infected with microsporidian spores.

Uninfected females produced significantly more eggs per mated pair than microsporidia-infected females (Table 7.1). Observed differences among the mean number of eggs produced per mated pair for the four mating combinations suggest that egg production is reduced when either males or females are infected. Although the results appear to represent a trend for decreased egg production when healthy females are mated with infected males, there was no significant difference between the mean number of eggs produced by uninfected females when mated with either uninfected or microsporidia-infected males (see Table 7.1). Similarly, there was no significant difference between the mean number of eggs produced for microsporidia-infected females when mated with either uninfected or microsporidia-infected males. Decreased egg production may be due to reduced fitness of both infected parents as a result of disease.

Regular performance testing of predators and routine examination of *P. persimilis* are essential for the management of *M. phytoseiuli* within commercial insectaries. Disease prevalence and transmission data have shown that this pathogen can become prevalent in the host population within a short period.

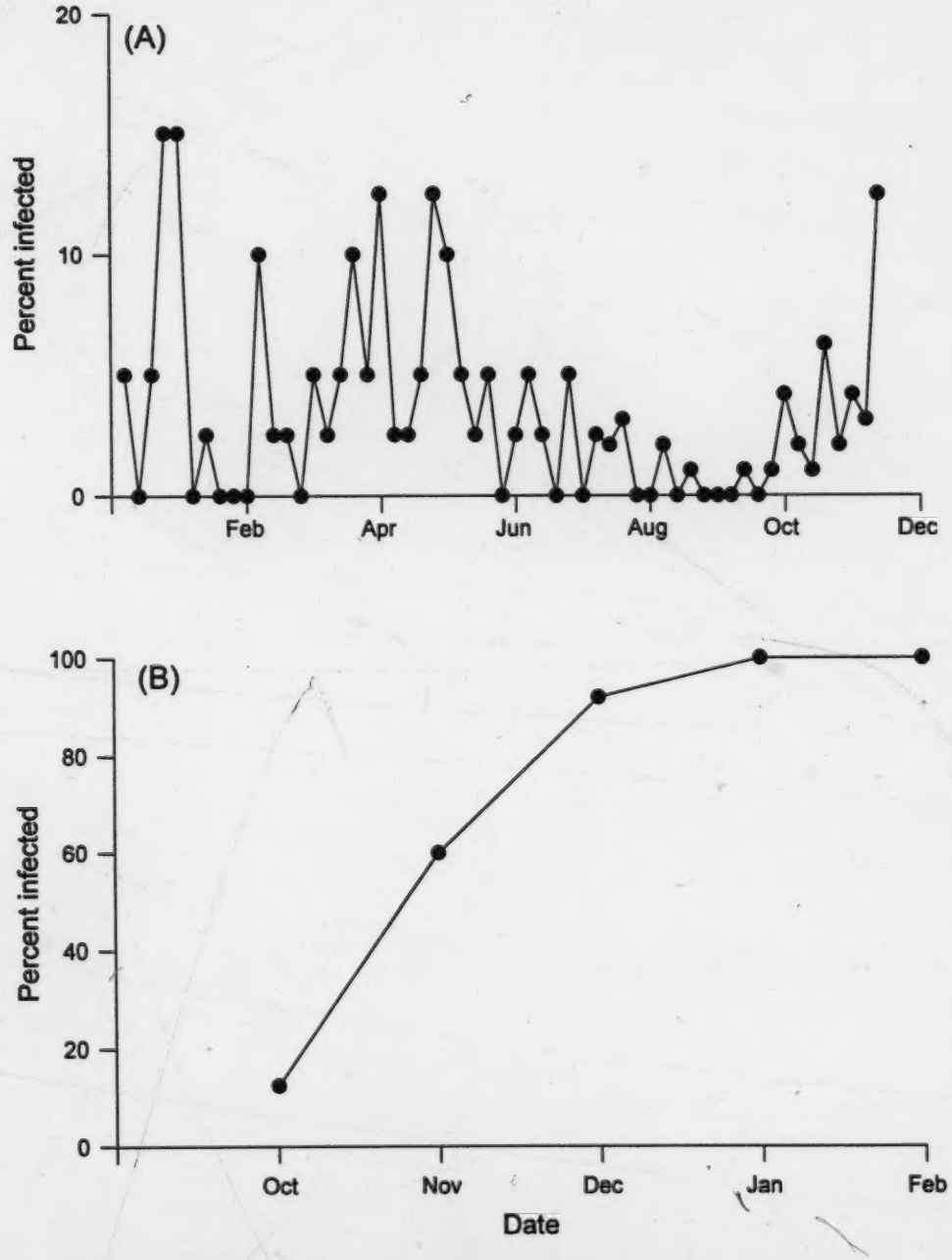

Figure 7.1 Disease prevalence of *Microsporidium phytoseiuli* in *Phytoseiulus persimilis* from Cage 1 (A) and Cage 2 (B). Individuals were sampled weekly from Cage 1 and monthly from Cage 2.

Table 7.1
Parental Contribution Toward Infection of *Phytoseiulus persimilis* Progeny with *Microsporidium phytoseiuli*

Infection status		Mated pairs	Eggs produced	Mean eggs produced	Eggs examined	Infected eggs
Female	Male	(n)	(n)	per mated pair	(n)	(%)
Uninfected	Uninfected	17	1075	63.2 a	444	0
Uninfected	Infected	16	833	52.1 a,b	630	0
Infected	Uninfected	18	563	31.3 c	251	100
Infected	Infected	18	706	39.2 b,c	270	100

Means followed by the same letter are not significantly different. Significance determined with a one-way ANOVA, Bonferroni multiple comparison test; ($F=10.518$; $P<0.001$; $df=3,65$).

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Chapter 8
Evaluation of cultural methods and chemical compounds for control of *Microsporidium phytoseiuli* in *Phytoseiulus persimilis*

Introduction

A primary goal within a commercial insectary is to provide optimal conditions for mass production of arthropods. When conditions within the rearing system are suboptimal, stress within populations may increase disease susceptibility (Goodwin, 1984). Some pathogens, including microsporidia, capable of producing sublethal or debilitating disease often remain undetected within arthropod populations. Microsporidia may be detected only through close monitoring of individuals within a colony. More noticeable effects in colony performance occur when disease prevalence is high (Goodwin, 1984). Once detected, a means for controlling microsporidia within the commercial insectary is needed.

Cultural methods used to control microsporidia associated with arthropod hosts include isolation techniques, heat treatments and egg-rinsing techniques. Pasteur (circa 1865) devised a method for excluding pathogens of silkworm by carefully screening eggs and adults and excluding those infected with microsporidia (Tanada and Kaya, 1993). Although a reliable method for maintaining disease-free stock, this method is labour intensive and suited only for colonies that initially consist of at least a few uninfected individuals. Heat treatments have been used successfully to eliminate the microsporidium *Nosema muscidifuracis* from the pteromalid parasitoid, *Muscidifurax raptor*. In this case, infected parasitoid eggs within fly puparia were immersed in a heated water bath (Geden *et al.*, 1995). Another approach is to rear infected individuals at elevated temperatures. This method has been used for control of microsporidioses in potato tuberworm, *Gnorimoschema operculella* Zeller (Allen and Brunson, 1947) and European corn borer, *Ostrinia nubilalis* (see Raun, 1961). When reared at elevated temperatures, sporulation of *Nosema heliothidis* within the silk glands of corn earworm (*Heliothis zea*) ceased. A reduction in transovarial transmission also permitted isolation of uninfected hosts that were used to establish a disease-free colony (Hamm *et al.*, 1971). Egg-rinsing techniques have been successful for controlling *Nosema* sp. that are transmitted *per os* in colonies of the mosquito

Anopheles stephensi Liston (Alger and Undeen, 1970).

Several chemical compounds have been investigated for their potential to control microsporidia in both invertebrate and vertebrate hosts. The benzimidazole compounds, including benomyl, metronidazole, and albendazole, inhibit tubulin formation and cause aberrant spore formation and disruption of the microsporidian life cycle (Lacey, 1990; Edlind *et al.*, 1996; Li *et al.*, 1996).

Benomyl has been incorporated into artificial diets and tested against microsporidia with variable results. Benomyl has eliminated microsporidia from alfalfa weevil larvae, *Hypera postica* Gyllenhal, without causing detrimental effects to the host (Hsiao and Hsiao, 1973); however, high host mortality was observed in *Drosophila willistoni* Sturtevant (Armstrong, 1976). Although benomyl suppressed microsporidia in eastern spruce budworm, *Choristoneura fumiferana*, it reduced growth, fertility and egg hatch of the host (Harvey and Gaudet, 1977). Benomyl was ineffective when used to control *Nosema heliothidis* in the corn earworm, *Heliothis zea* (see Brooks *et al.*, 1978). No generalisation can be made about the effectiveness of benomyl for control of microsporidia in insects.

Of all benzimidazole compounds tested for their potential to control microsporidiosis, metronidazole appears to be least effective. Metronidazole has been used to alleviate diarrhea caused by *Enterocytozoon bieneusi* Desportes, Le Charpentier, Galian, Bernard, Cochand-Priollet, Lavergne, Ravisse and Modigliani in human AIDS patients, but was ineffective at eliminating the microsporidium (Blanshard *et al.*, 1992). In another study, spore germination in cell culture of the vertebrate pathogens *Encephalitozoon hellem* E.S. Didier, P.J. Didier, Frieberg, Stenson, Orenstein, Yee, Tio, Davis, Vossbrink, Millichamp and Shadduck and *E. intestinalis* Cali, Kolter and Orenstein was weakly inhibited by metronidazole (He *et al.*, 1996) but Moffett *et al.* (1969) found metronidazole administered in sugar syrup solution ineffective for control of *Nosema apis* in the honeybee, *Apis mellifera*.

Based on results from a variety of past studies, albendazole seems a more promising candidate for controlling microsporidia than either benomyl or metronidazole. Albendazole has been used to control diarrhea and wasting

syndrome associated with intestinal microsporidioses in human AIDS patients (Blanshard *et al.*, 1992; De Groote *et al.*, 1995; Didier *et al.*, 1996; Joste *et al.*, 1996). In one study, albendazole treatment eliminated microsporidian spores from human faeces in autopsy examinations with no evidence of residual microsporidiosis in major organs (Joste *et al.*, 1996).

When added to *Spodoptera frugiperda* Smith cells *in vitro*, albendazole caused a significant reduction in cells infected with *Nosema bombycis* and established infections of *N. bombycis* were almost eliminated from 6th-instar *Heliocoverpa* (*Heliothis*) *zea* larvae and pupae without deleterious effects to the host (Haque *et al.*, 1993).

Aside from the benzimidazoles, few other compounds have been used successfully as antimicrosporidial agents. Fumagillin, a compound derived from the fungus *Aspergillus fumigatus* Fresenius, was recently proven effective as a topical treatment for microsporidial keratoconjunctivitis in a human AIDS patient (Garvey *et al.*, 1995). In invertebrate hosts, fumagillin has been used in water or sugar solutions to suppress microsporidioses in the honey bee, *Apis mellifera* (see Katznelson and Jamieson, 1952; Bailey, 1953; Moffett *et al.*, 1969) and *Drosophila willistoni* with minimal detrimental effects on survival (Armstrong, 1976). Incorporation of fumagillin in artificial diets reduced disease within alfalfa weevil (*Hypera postica*) larvae (Hsiao and Hsiao, 1973) with no apparent deleterious effects to the host. In another study, incorporation of fumagillin into diet reduced disease within boll weevil (*Anthonomus grandis* Boheman) colonies but reduced host fecundity and egg hatch (Flint *et al.*, 1972). Treatment of European corn borer larvae, *Ostrinia nubilalis*, with fumagillin in artificial diet reduced the severity of infection of the microsporidium *Perezia pyraustae* Paillot (Lewis and Lynch, 1970); however, the disease reoccurred once the drug was withdrawn (Lynch and Lewis, 1971). Fumagillin applied as a solution of sugar water was ineffective for control of *Nosema muscidifuracis* in *Muscidifurax raptor* (see Geden *et al.*, 1995). Other agents tested include toltazuril, an anti-coccidial drug, which has been used to control microsporidiosis in insects and fish (Melhorn *et al.*, 1988; Schmahl *et al.*, 1990) and nifedipine, a calcium channel blocker, which inhibits

germination of *Encephalitozoon hellum* spores isolated from human AIDS patients (He *et al.*, 1996).

The aim of this study was to investigate both cultural and chemical methods for control of microsporidia in mass-reared *Phytoseiulus persimilis*. Cultural methods evaluated for control included pathogen exclusion (Pasteur method) and heat treatment. Topical applications of antimicrosporidial compounds, including albendazole, fumagillin, nifedipine and metronidazole, were evaluated for their potential to control microsporidia in *P. persimilis*.

Materials and Methods

Pasteur method The original shipment of *P. persimilis* from source EU1 consisted of both uninfected and microsporidia-infected predators. The Pasteur method of isolating and removing infected individuals was used to establish uninfected and microsporidia-infected colonies.

Gravid females were randomly selected, placed on excised leaf discs within observation dishes (described in Chapter 6 and Appendix 4), and allowed to produce eggs over a period of 24 to 72 hr. Eggs removed after the first 24 hr of isolation were smeared, stained in 15% Giemsa buffer and examined for microsporidian spores. These eggs were used to establish the infection status of each isolated female. Eggs produced during the last 48 hr of the observation period were left on leaf discs and each adult female was removed, smeared and examined for microsporidian spores. Eggs produced by uninfected and infected predators were then used to establish separate colonies of uninfected and infected predators, respectively. Each colony was isolated and maintained under quarantine conditions; the colony established from uninfected predators was maintained pathogen-free from December 1993 until June 1996.

Uninfected predators used in the following two studies were obtained from a European source (EU2) and compared with microsporidia-infected mites from source EU1 (see Chapter 6 for details of colony history and maintenance).

Rearing Phytoseiulus persimilis at 30°C Uninfected and microsporidia-infected gravid females were randomly selected and isolated within observation dishes. These were placed within a larger plastic container within an incubator

(16L:8D; 30°C). After 24 hr, female predators were removed from dishes and examined for microsporidian spores. All eggs produced during this period were permitted to hatch and develop for 10 days at 30°C. Progeny were examined daily and permitted to mate with their siblings.

Progeny were fed twice during the experimental period by removing the older leaf disc (with predators) and inverting it on a fresh disc, so that both lower leaf surfaces were in contact. Care was taken to ensure that progeny were not crushed. Older discs remained in observation dishes for 24 hr and were removed when progeny were next examined. This process was a more efficient means of transferring predators and although most predators moved onto the fresh disc on their own, dishes were examined thoroughly to ensure that all eggs and progeny had been transferred. At the end of the study period, progeny were smeared, categorized by developmental stage (egg, immature, adult) and examined for microsporidian spores.

Chemical applications Uninfected and microsporidia-infected *P. persimilis* females were randomly selected and isolated on excised leaf discs within observation dishes. Eggs were removed after 24 hours and placed within depressions on microscope slides using a fine bristled paint brush. Female predators were then examined to confirm infection status.

Treatments *Phytoseiulus persimilis* has not been reared on artificial diets, therefore topical applications of antimicrosporidial compounds were used. All compounds (albendazole, fumagillin, nifedipine and metronidazole) were mixed in dimethyl sulfoxide (DMSO) and applied directly to eggs in the following three concentrations: 1000 μg/mL, 100 μg/mL and 10 μg/mL. Each chemical concentration was made by serial dilution of a 100 mg/mL stock solution and some microsporidia-infected eggs were treated with this solution. Eggs treated with DMSO served as a control.

Eggs were treated for 60 sec; excess solutions were blotted away with filter paper. Eggs were then transferred onto spider mite-infested leaf discs in observation dishes using a fine bristle paint brush (10 eggs per dish). Observation dishes were placed in plastic containers within an incubator (16L:8D; 25°C:20°C).

Eggs were examined daily and those that desiccated within the first 24 hr of the test period were not included in data analysis. Egg hatch was recorded and progeny that hatched from uninfected and microsporidia-infected eggs were allowed to develop for six and eight days, respectively, before being examined for microsporidian spores. Eggs produced by progeny during the study period were smeared and examined for microsporidian spores.

Results

Pasteur method Separate colonies of uninfected and microsporidia-infected predators were established using the Pasteur method. Isolated colonies were each maintained under quarantine conditions. The colony established from uninfected predators was maintained pathogen-free until June 1996. Despite efforts to maintain disease-free status in the uninfected colony, all predators from source EU1 eventually became infected with microsporidia (see Chapter 6).

Rearing Phytoseiulus persimilis at 30°C After 10 days at 30°C, uninfected females had produced three times as many progeny as microsporidia-infected predators (Table 8.1). Uninfected females (n=35) produced 128 progeny over the test period and microsporidian spores were not observed in any of the 115 progeny examined. In contrast, microsporidia-infected *P. persimilis* females (n=35) produced 41 progeny; all 35 progeny examined were infected with microsporidian spores.

Chemical applications Effects of albendazole, fumagillin, metronidazole and nifedipine on egg hatch and control of microsporidiosis when applied as a solution in DMSO are shown in Table 8.2. For all treatments, no distinct trend in egg hatch was apparent. Some eggs failed to hatch due to desiccation; desiccated eggs often turned from light yellow to dark gold and became rubber-like in texture.

Uninfected eggs (EU2) Egg hatch was observed after all treatments at all doses. Larval mortality was observed following treatment with 1 mg/mL and 100 μg/mL albendazole or nifedipine. Microsporidian spores were not observed in smear preparations of progeny from uninfected females, nor were spores observed in eggs produced by these progeny.

Microsporidia-infected eggs (EU1) Although eggs collected from microsporidia-infected predators did not hatch when treated with 100 mg/mL albendazole, egg hatch was observed following treatments of 100 mg/mL fumagillin, metronidazole and nifedipine. Larval mortality (observed following most doses of all four treatments) was frequently observed following treatment of microsporidia-infected eggs. All progeny that hatched from infected eggs contained microsporidian spores regardless of treatment or dose. All eggs produced by progeny during the test period contained microsporidian spores.

Discussion

Pasteur method The Pasteur method can be used to discriminate between uninfected hosts and those infected with pathogens that cause no external signs or symptoms, including *M. phytoseiuli*. Hosts can be isolated and their health determined indirectly through the examination of their eggs or progeny, or the parent can be examined directly after several progeny have been produced. Pathogen exclusion may be the most reliable method for controlling microsporidia and other pathogens; this method was the only dependable means to eliminate microsporidia from *P. persimilis* colonies.

The Pasteur method is a suitable means to control microsporidia while disease prevalence within a colony remains low. Once disease becomes highly prevalent within a population, isolation and examination of hosts for pathogens can become time-consuming. As disease prevalence increases, many hosts must be screened to find the few uninfected hosts. Under such conditions, it is difficult to isolate enough uninfected hosts to form a new uninfected colony with a diverse genetic background.

Rearing Phytoseiulus persimilis at 30°C Temperatures for optimal development of *P. persimilis* range from 21°C to 27°C (Steiner and Elliott, 1987) and predators do not thrive at temperatures above 30°C (Scopes, 1985). When reared at 30°C, uninfected *P. persimilis* females that survived in this study produced three times as many progeny as microsporidia-infected predators. All progeny produced by infected females were infected with microsporidian spores. Based on these results, rearing *P. persimilis* above temperatures for optimal

development is an impractical approach for eliminating microsporidia from infected hosts directly.

Rearing predators at elevated temperatures may prove useful for controlling microsporidian pathogens within a predator colony when disease prevalence is low. Higher temperatures (30°C and above) may indirectly reduce microsporidia from the colony by killing infected hosts or reducing their reproductive potential. Results demonstrate that uninfected predators produce more progeny than infected predators when reared at elevated temperatures and this may contribute to lower disease prevalence within the colony.

Temperatures above 30°C are not uncommon in greenhouses during the growing season. Although microsporidia were not eliminated from *P. persimilis* when reared at 30°C, results suggest that high temperatures may hinder the reproductive success of microsporidia-infected predators; however, effects of elevated temperatures on the performance of infected *P. persimilis* in the greenhouse have not been quantified.

Chemical applications Albendazole, fumagillin, metronidazole, and nifedipine were ineffective for control of microsporidia in *P. persimilis*, regardless of dose. Although all treatments were expected to be toxic at doses of 100 mg/mL, some eggs hatched when treated with 100 mg/mL fumagillin, metronidazole and nifedipine. Although DMSO is recognised for its unique ability to penetrate surfaces and enhance absorption of compounds (see Merck Index, 1976), results of this experiment raise doubt as to whether the chemical compounds used in this study were able to penetrate the egg chorion. DMSO may be unable to bind to these chemicals to transport them across the chorion of the egg or may inactivate them prior to transport.

Although some chemical compounds, including fumagillin and benomyl, used in past studies suppress microsporidia, reoccurrence of the pathogen occurred once the drug was withdrawn (Lynch and Lewis, 1971; Hsiao and Hsiao, 1973). It is possible that chemicals investigated for their antimicrosporidial properties control only a portion of the microsporidian life cycle and may be most effective when used concurrently with other drugs. For example, He *et al.* (1996)

found nifedipine and metronidazole more effective for the inhibition of microsporidian spore germination when used together than when each was used independently.

General conclusions The Pasteur method of isolating and removing infected individuals was the most reliable means for controlling microsporidia in this study. Rearing microsporidia-infected *P. persimilis* at temperatures above those for optimum development resulted in high mortality and was ineffective at eliminating the pathogen. This approach may prove effective for reducing microsporidia from a colony that consists of both uninfected and microsporidia-infected individuals, whereby increased mortality rates of infected hosts would result in an overall decrease in infection rate. Even so, individuals must be examined during and after rearing at elevated temperatures and since this is the basis of the Pasteur method, the latter is a more time-efficient and dependable approach for removing infected individuals from a colony. Furthermore, isolation and removal of infected individuals using the Pasteur method does not require the use of an incubator or other specialised equipment.

Chemical treatments of albendazole, fumagillin, metronidazole, and nifedipine were ineffective at controlling microsporidia in *P. persimilis*. Because *P. persimilis* has not been reared with success on artificial diet, an effective means to administer antiprotozootics remains the largest obstacle for chemical treatment of microsporidia in this predator. Chemical treatments cannot be successfully administered to the predator via the prey. Phytophagous mites, including *Tetranychus urticae*, feed by piercing plant foliage and extracting plant fluids. Under such circumstances, chemical compounds must be applied to plants at higher concentrations than would be required if applied directly to prey or predatory mites. With this approach, dosages cannot be controlled and chemical treatments may prove to be phytotoxic.

Table 8.1
Effect of Elevated Rearing Temperature (30°C) on Infection of *Phytoseiulus persimilis* Progeny

	Parent ♀s (n)	Number of progeny produced†			Total progeny	Number examined	Percent infected
		Eggs	Immatures	Adults			
Uninfected	35	42	60	26	128	115	0
Infected	35	12	11	18	41	35	100

† number of progeny produced at 30°C after 10 days.

Table 8.2
Treatment of Uninfected and Microsporidia-infected *P. persimilis* Eggs with Albendazole, Fumagillin, Metronidazole and Nifedipine

							Progeny					Progeny eggs	
Source	Treatment	Dose	n	Eggs (dsc)	Eggs (dnh)	Eggs (n)	Female	Male	Immatures (dead)	Hatch (%)	Infected (%)	n	Infected (%)
Uninfected (EU2)	albendazole	1 mg/mL	11	2	3	5	4	0	2	54.5	0	0	
		100 μg/mL	11	0	0	0	9	1	1	100	0	0	
		10 μg/mL	8	0	4	4	3	1	0	50.0	0	0	
		DMSO	11	0	0	0	6	5	0	100	0	0	
	fumagillin	1 mg/mL	8	2	1	3	3	2	0	62.5	0	2	0
		100 μg/mL	8	0	0	0	6	2	0	100	0	6	0
		10 μg/mL	6	3	0	3	2	1	0	50.0	0	0	
		DMSO	6	3	0	3	1	2	0	50.0	0	0	
	metronidazole	1 mg/mL	13	0	0	0	5	8	0	100	0	0	
		100 μg/mL	16	3	3	6	7	3	0	62.5	0	1	0
		10 μg/mL	17	5	4	9	7	1	0	47.1	0	0	
		DMSO	16	7	0	7	5	4	0	56.3	0	0	
	nifedipine	1 mg/mL	12	1	1	2	5	3	2	83.3	0	0	
		100 μg/mL	13	3	1	4	6	1	2	69.2	0	0	
		10 μg/mL	17	3	6	9	5	3	0	47.1	0	0	
		DMSO	17	5	0	5	8	4	0	70.1	0	0	

Eggs (dsc), desiccated; Eggs (dnh), did not hatch.

Table 8.2 (continued)
Treatment of Uninfected and Microsporidia-infected *P. persimilis* Eggs with Albendazole, Fumagillin, Metronidazole and Nifedipine

							Progeny					Progeny eggs	
Source	Treatment	Dose	n	Eggs (dsc)	Eggs (dnh)	Eggs (n)	Female	Male	Immatures (dead)	Hatch (%)	Infected (%)	n	Infected (%)
Infected (EU1)	albendazole	100 mg/mL	16	3	13	16	0	0	0	0		0	
		1 mg/mL	43	21	10	31	4	7	1	28.0	100	0	
		100 μg/mL	31	13	6	19	3	6	3	38.7	100	0	
		10 μg/mL	38	14	0	14	11	8	5	63.2	100	15	100
		DMSO	30	12	2	14	6	10	0	53.3	100	12	100
	fumagillin	100 mg/mL	18	10	0	10	3	3	2	44.4	100	0	
		1 mg/mL	39	12	6	18	13	7	1	53.8	100	43	100
		100 μg/mL	39	11	5	16	13	10	0	59.0	100	38	100
		10 μg/mL	44	10	8	18	17	9	0	59.1	100	75	100
		DMSO	35	17	1	18	5	11	1	48.6	100	14	100
	metronidazole	100 mg/mL	20	10	1	11	7	2	0	45.0	100	0	
		1 mg/mL	33	11	5	16	9	7	1	51.5	100	0	
		100 μg/mL	36	12	3	15	5	12	4	58.3	100	8	100
		10 μg/mL	34	12	0	12	13	4	5	64.7	100	7	100
		DMSO	35	13	2	15	6	9	5	57.1	100	2	100
	nifedipine	100 mg/mL	16	8	0	8	2	3	3	50.0	100	0	
		1 mg/mL	37	20	0	20	8	8	1	46.0	100	16	100
		100 μg/mL	33	17	2	19	5	8	1	42.4	100	10	100
		10 μg/mL	45	14	4	18	16	10	1	60.0	100	19	100
		DMSO	35	15	4	19	8	6	2	45.7	100	3	100

Eggs (dsc), desiccated; Eggs (dnh), did not hatch.

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Chapter 9
Conclusions

Morphological observations of P. persimilis Despite the economic importance of *Phytoseiulus persimilis*, few studies have been undertaken to investigate the internal and external morphology of this acarine predator. Hessein (1976) described external morphology and provided illustrations of both dorsal and ventral shields and arrangements of setae for all developmental stages. Akimov and Yastrebtsov (1987) described the gnathosomal, pharyngeal and opisthosomal muscles and their attachment sites. In other studies, only the digestive system of *P. persimilis* has been investigated in any detail (Akimov and Starovir, 1974, 1978). With few exceptions, illustrations used in previous studies to portray morphological and anatomical features of *P. persimilis* are based on light microscopic observations. Although these studies provide useful morphological observations, resolution of detail is often inadequate and diagrams are often difficult to interpret at this level of resolution.

The aim of this study was to describe normal *P. persimilis* tissues and internal anatomy so that comparisons with internal anatomy and tissues of diseased mites could be made. The internal morphology of *P. persimilis* adult females was described during this study; however, further work is required to confirm the identification of what were described in this study as digestive cells. Although these cells bear a resemblance to those reported in earlier studies as digestive cells (Starovir, 1973; Akimov and Starovir, 1974, 1978), histological evidence suggests that they are likely a portion of the lyrate organ described by Michael (1892). Further investigation is required to provide more detailed information on the female reproductive system and additional information regarding reproductive tissues and accessory glands of male *P. persimilis*. Future studies should include a description of the internal anatomy of immatures as well as adults. Increased knowledge of the internal anatomy of immatures would contribute toward an increase in our current understanding of tissue development in this predator and its role in disease development.

Abdominal discolouration and rectal plug formation Disease signs observed

during this study included abdominal discolouration and rectal plug formation. In affected mites, abdominal discolouration was manifested as either two white stripes down the lateral sides of the body in the region of the Malpighian tubules or as a U-shaped discolouration of the distal opisthosoma. Disease signs were attributed to the accumulation of birefringent dumbbell-shaped crystals (Bjørnson *et al.*, 1997); however, the cause of crystal accumulation is unknown.

Other researchers have attributed abdominal discolouration in mass-reared mites to senescence (Tanigoshi, 1982) or pathogens (Schütte *et al.*, 1995); however, these theories are not supported by information collected here. Although birefringent crystals were observed in *P. persimilis* from all 14 sources examined, occurrence of crystals was not correlated to the presence of rickettsia, virus-like particles or microsporidia observed during my study (Bjørnson *et al.*, 1997). Observations of abdominal discolouration and rectal plug formation appear consistent with poor predator performance. Affected *P. persimilis* often appeared lethargic (Bjørnson *et al.*, 1997) and excessive crystal accumulation has been reported to be responsible for poor response to herbivore-induced synomones (Schütte *et al.*, 1995).

Observations of abdominal discolouration and rectal plug formation in *P. persimilis* were more common among individuals when overcrowding contributed toward temporary starvation. Observations by Hughes (1950) indicated that other factors may contribute toward disease signs, including host plant nutrition and environmental factors such as fluctuations in temperature and length of daylight. Abdominal discolouration and rectal plug formation of *P. persimilis* remain important issues within the biological control industry. Future research is required to define the precise cause of abdominal discolouration in *P. persimilis* and formulate practical solutions to reduce these effects and increase predation efficacy.

Microsporidia and P. persimilis Microsporidia were described from *P. persimilis* obtained from three commercial sources: one each from North America, Europe and Israel. Twenty-seven shipments of *P. persimilis* were received from the North American source from November 1990 to February 1993; seven of these

were infected with microsporidia. One shipment of *P. persimilis* was received from both European and Israeli sources and predators from both shipments were infected with microsporidia. Based on ultrastructural observations, microsporidia described from *P. persimilis* from these sources appeared to represent three distinct species. Although sources of these microsporidia are unknown, it seems reasonable to conclude that these pathogens are endemic to the regions from where *P. persimilis* were mass-produced.

Microsporidia were also observed within *P. persimilis* from a second North American source and two Australian sources (Bjørnson *et al.*, 1997). Resin-embedded *P. persimilis* from the second North American source were poorly preserved and microsporidia could not be adequately described from these specimens. Microsporidia from the Australian source were observed only by light microscopy and no live or prepared specimens were available for examination by TEM. Therefore, it could not be determined whether microsporidia in *P. persimilis* from these two sources represent two additional microsporidian species or were similar to those reported in this study.

The occurrence of more than one microsporidian species in *P. persimilis* obtained from different commercial sources suggests that these pathogens are endemic to the area where the host is mass-reared. Insectaries that exchange predators for the purpose of preventing genetic bottlenecks within mass-reared colonies may also acquire one or more species of microsporidia. Studies of disease prevalence, host effects and parasite transmission of *Microsporidium phytoseiuli* suggest that microsporidia can pose a threat to successful establishment of mass-rearings of *P. persimilis*.

Addition of field-collected *P. persimilis* to commercial mass-reared stock may prove to be an important management tool for reducing parasitism by microsporidia. This practice helps maintain high genetic diversity within mass-reared populations when carried out on a routine basis. However, predators collected from the field for colonisation purposes may also be infected with microsporidia and individuals should be examined to ensure disease-free status prior to their addition to insectary mass-rearings. Without routine examination of

predators, pathogens may continue to be unwittingly distributed to insectaries and commercial growers.

Effects of microsporidia on predator performance *Microsporidium phytoseiuli* caused significant reductions in short-term oviposition rates and prey consumption of *P. persimilis* adult females. Lifetime oviposition rates and longevity of infected females were also significantly reduced. These effects were observed when predators were subjected to controlled conditions (25°C:20°C; 16L:8D); however, effects of microsporidia on predator performance may be more pronounced within commercial greenhouses where conditions are less stable. Diseased predators may be unable to withstand stresses such as fluctuating environmental conditions, low prey densities, and temporary starvation.

The effects of stress, including overcrowding, sub-optimal nutrition, and temperature extremes on hosts with chronic microsporidiosis have not been fully investigated (Maddox, 1987). Such stresses are often associated with the insectary environment and may contribute toward an increase in disease susceptibility of individuals and an overall increase in disease prevalence within the predator population. Numerous reports of previously undescribed pathogens in mass-produced insects within insectaries and laboratories support this hypothesis.

Microsporidia have been reported to infect numerous biological control agents and natural enemies and many microsporidian species have been found to cause detrimental affects to their respective hosts: these include decreased fecundity and survivorship, increased mortality and aberrant host development. In my study, only *Microsporidium phytoseiuli* was investigated for its effects on *P. persimilis*; however, due to past reports of microsporidia and their effects on other natural enemies, it seems reasonable to conclude that other microsporidia that infect *P. persimilis* are also likely to cause detrimental effects to this predator.

Poor performance of microsporidia-infected *P. persimilis* may go unnoticed in commercial greenhouses unless predators are closely monitored on a routine basis after release. Growers who depend on *P. persimilis* may discover unsatisfactory control only when crop damage has become extensive. A rapid increase of the pest population and extensive crop damage may leave growers with

few control options and the application of chemical compounds translates to additional time and money spent on pest control. Use of chemicals for pest control after biological control agents have been released may kill other natural enemies that have become established. Use of microsporidia-infected predators may ultimately reduce grower confidence in the use of *P. persimilis* and other natural enemies.

In addition to performance problems, microsporidia-infected *P. persimilis* adult females produced significantly fewer female progeny than uninfected predators. The sex ratio of *P. persimilis* in previous studies has been reported to range from 3.8:1 to 4.6:1 (Kennett and Caltagirone, 1968; Laing, 1968; Takafuji and Chant, 1976) and sex ratio alterations may have considerable impact on establishment and reproductive success of *P. persimilis* both in commercial greenhouses and when released in the field.

Although microsporidia are known to cause sex ratio distortions in arthropods including the brackish water shrimp, *Gammarus duebeni* Lilljeborg (Dunn *et al.*, 1993), microsporidia have not been fully investigated for their potential to significantly alter the sex ratio of insects or mites. In one study, the sex ratio of *Trichogramma nubilale* Ertle and Davis adults were not significantly altered when infected with the microsporidium *Nosema pyrausta* (see Saleh *et al.*, 1995). Further information regarding sex ratio alterations due to microsporidia is required to assess the impact that these pathogens could have on the reproductive success of their arthropod hosts.

Disease prevalence and transmission Symptoms of reduced prey consumption, oviposition rates, and longevity of microsporidia-infected *P. persimilis* become more problematic when considered in association with the ability of *M. phytoseiuli* to become highly prevalent within the host population over a short period. Disease prevalence of *M. phytoseiuli* in *P. persimilis* from an established colony remained below 15 percent over a one year period, only to increase to 100 percent over two months at the end of the sampling period. Due to low disease prevalence, *M. phytoseiuli* spores were not always observed in examined predators, despite sample sizes of almost 100 predators per week. This

suggests that performance tests that employ smaller sample sizes on a seasonal basis, as recommended in current quality control guidelines (van Lenteren, 1994), are unlikely to contribute toward successful detection of pathogens, even if recommendations are revised to include examination of individual predators for pathogens.

Horizontal transmission remains poorly understood in this system. Spores present in immatures (light microscopy) and in adults (TEM) indicate the ability of the pathogen to replicate in these stages. Horizontal transmission, however, was only successful when uninfected immatures were permitted to develop in proximity to infected immatures and adults. It is possible that immature predators are more susceptible than adults to infection by microsporidia. Infection of specific tissues, such as the lyrate organ, in early development of the immature may be necessary for successful vertical transmission from adult female to her progeny.

Microsporidium phytoseiuli is a microsporidium with low horizontal and high vertical transmission rates. These characteristics permit isolation of uninfected predators when disease prevalence is low. Progeny from uninfected predators can be used to reestablish uninfected colonies. This process becomes more difficult and time-consuming once disease prevalence increases and more complicated by the observation that at least three species of microsporidia infect *P. persimilis* (see Bjørnson *et al.*, 1996). Due to similarities in spore size and shape, it is difficult to distinguish *M. phytoseiuli* from other microsporidia without examination by TEM and it cannot be assumed that all microsporidia will be transmitted in a similar manner.

Despite efforts to isolate uninfected *P. persimilis* and establish a colony from uninfected progeny, all individuals can become infected after several months of quarantine. Difficulties in maintaining *P. persimilis*, and other biological control agents, free of microsporidia can become even more complicated within a commercial insectary where conditions are less controlled and quarantine areas may be unavailable. Even when a few individuals within mass-rearings are infected with an undetected pathogen, disease can quickly become prevalent due

to overcrowding of laboratory and insectary-reared colonies (Geden *et al.*, 1995).

Although antimicrosporidial compounds have been used to successfully control microsporidia in a variety of invertebrate and vertebrate hosts, use of albendazole, fumagillin, metronidazole or nifedipine did not control *M. phytoseiuli* in *P. persimilis*. It is possible that these compounds were unable to penetrate the chorion of the egg; however, it is also possible that these antimicrosporidial compounds provide only temporary control of microsporidiosis, perhaps by affecting the vegetative portion of the microsporidian life cycle while having no effect on microsporidian spores. Although antimicrosporidial agents have been reported to reduce microsporidiosis in some cases, the host may become reinfected after these compounds are withdrawn (Lynch and Lewis, 1971).

Repeat treatments of antimicrosporidial compounds may be required to provide adequate control of microsporidia. Some researchers have reported success at reducing spore germination with the simultaneous use of more than one antimicrosporidial compound, each with a different mode of action (He *et al.*, 1996). Further investigation is required to find an effective and practical means to control microsporidia in mass-rearings.

Value attributed to the use of disease-free P. persimilis Microsporidia-infected *P. persimilis* produced significantly fewer eggs and consumed significantly fewer prey than uninfected predators. Uninfected and microsporidia-infected females produced 2.53 and 1.61 eggs per female per day, respectively, during prey consumption studies (when tested over five days). Therefore, 1.57 microsporidia-infected adult female predators are required to produce as many eggs as one uninfected adult female.

Number of prey consumed per egg produced was slightly lower for uninfected (7.41:1) than infected adult females (8.31:1) during the five day prey consumption study. This suggests that food conversion is more efficient for uninfected predators than microsporidia-infected predators. During prey consumption studies (five days), uninfected and microsporidia-infected adult females consumed 18.74 and 13.39 prey deuteronymphs, respectively. Therefore, 1.4 microsporidia-infected adult female predators are required to consume as

many prey as one uninfected adult female.

These values suggest that 1.4 to 1.57 microsporidia-infected female predators are required to produce as many eggs and/or consume as many prey as uninfected female predators. Based on these estimates, use of microsporidia-infected predators for pest control could result in added expenditures of 50 percent. These additional costs may be underestimated because microsporidia-infected predators do not live as long as uninfected predators and performance may be further affected by unstable greenhouse environments and sex ratio alterations. Additional shipping and application costs must also be considered.

Mass-rearing, quarantine and routine examination Although considerable care is taken to exclude predators and hyperparasitoids from biological control candidates prior to their mass production and release, inadequate attention may be given toward elimination of disease. Many biological control candidates are maintained under quarantine for several months and one or more generations before release into the field. This technique may be satisfactory for highly virulent pathogens but inadequate for those causing chronic diseases. Without rigorous screening and microscopic examination to detect pathogens, results from performance tests can only provide inconclusive information regarding quality of predators and parasitoids. Arthropods mass-reared under suboptimal conditions in laboratories or insectaries are often more susceptible to disease (Goodwin, 1984; Maddox, 1987), yet many pathogens remain undetected in mass-rearings because of the chronic diseases that they cause (Goodwin, 1984). The ability of *M. phytoseiuli* to become prevalent within laboratory-reared colonies of *P. persimilis* over a short period illustrates the importance of routine microscopic examination for detection of microsporidia at low disease prevalence.

Pathogens and the biological control industry More than 80 species of natural enemies are currently being reared in Europe, fewer species are available in North America (van Lenteren *et al.*, 1997). Few of these natural enemies have been investigated for pathogens and these were examined only after poor performance was noticed.

The microsporidium *Nosema muscidifuracis* was reported from the

pteromalid parasitoid *Muscidifurax raptor*, used for control of muscoid flies in agricultural settings (Becnel and Geden, 1994). Microsporidiosis in *M. raptor* resulted in longer developmental periods and production of fewer progeny (Zchori-Fein *et al.*, 1992). Microsporidia reported in the egg parasitoid *Trichogramma evanescens* Westwood (Huger, 1984) caused significant reductions in fecundity. Microsporidia have been reported to infect mass-reared *Neoseiulus* (formerly *Amblyseius*) *cucumeris* and *Amblyseius barkeri*, two acarine predators used to control western flower thrips (*Frankliniella occidentalis*) and onion thrips (*Thrips tabaci*), respectively (Beerling and van der Geest, 1991; Steiner, 1993a). Microsporidia in mass-rearings of these predators were suspected to reduce predator quality. Unidentified microorganisms reported from the spider mite predator *Metaseiulus occidentalis* were thought associated with rectal plug formation and reduced fecundity (Hess and Hoy, 1982). Rickettsia, virus-like particles and microsporidia have been reported in *P. persimilis* (see Šuťáková, 1988; Steiner, 1993a); however, only microsporidia have been investigated for their effects on predator performance. Rickettsia reported by Steiner (1993a) belong to the Genus *Wolbachia*, a rickettsial group known to cause sub-lethal effects to their hosts. These effects have not been quantified, however, and effects of virus-like particles observed within the yolk of developing eggs have not been studied.

Recent performance problems and reports of pathogens in mass-reared arthropods have raised concern regarding quality of biological control agents used for commercial pest control. Several publications have either focused attention on performance problems (Steiner, 1993a; Steiner 1993b: Steiner and Bjørnson, 1996) or addressed technical aspects of maintaining high quality production (Chambers and Ashley, 1984; Goodwin, 1984; Leppla and Fisher, 1989; van Lenteren, 1993a). The overall result has been the recommendation of quality control guidelines for more than 20 greenhouse biological control agents (van Lenteren, 1993b; van Lenteren, 1994). Despite increasing reports of microsporidia and other pathogens in mass-reared arthropods, quality control recommendations fail to promote examination of individuals for pathogens. It is difficult to determine if tests are reliable indicators of quality without considering pathogens and their effects on

host performance.

Prospects for the future The study of pathogens affecting biological control agents remains a relatively unexplored area of research. Most mass-produced natural enemies have not been investigated for potential pathogens. Future research is required to identify potential pathogens and their effects on natural enemies before poor performance is detected. Microsporidia have been reported in the stored product mites *Acarus siro* and *Tyrophagus putrescentiae* (see Beerling *et al.*, 1993; Larsson *et al.*, 1997), mites used as an alternative food source for rearing the thrips predators, *Amblyseius cucumeris* and *A. barkeri*. Future research is required to determine if microsporidia-infected *A. siro* and *T. putrescentiae* contribute toward the infection of natural enemies in mass-rearings.

Abdominal discolouration and rectal plug formation of *P. persimilis* has remained an important problem and further research is required to determine the precise cause of these disease signs and improve predator performance. Although rickettsia and virus have been described in mass-reared *P. persimilis*, the effects of these pathogens have not yet been investigated. Further investigation is required to determine the frequency and effects of several potential pathogens previously reported from other mass-reared natural enemies. Rickettsia and viruses were detected by TEM in previous studies and further work is required to develop rapid and reliable detection methods for these and other potential pathogens.

Realistic performance standards must include mandatory examination of individuals for pathogens on a routine basis. Regular performance testing must ensure that growers receive only disease-free insectary stock that are capable of performing within recommended standards. An impartial institution should be established and personnel with expertise in areas of invertebrate pathology and biological control should be responsible for pathogen identification, routine performance testing of natural enemies and the recommendation of realistic performance standards for mass-reared natural enemies. This work should be done in collaboration with producers and growers. Addressing issues of pathogens and their effects on mass-reared natural enemies will help renew and strengthen grower confidence in the use of greenhouse biological control agents.

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Appendix 1
General rearing methods

Bean plants Green beans (*Phaseolus vulgaris)* were used to rear pest mites and predators, and excised discs cut from bean foliage were used in all observational studies. Beans were seeded weekly (6 seeds/pot) in Metro-Mix 290 growing media (Grace Horticultural Products, Grace and Co. of Canada, Ltd.) within six inch round fibre pots. These were grown in a Conviron controlled environment chamber (25°C:20°C; 16L:8D) for three to four weeks. Relative humidity was not controlled but ranged from 70 to 90 percent. Plants were fertilized with 20-20-20 at a rate of 5.2g/4.5L once each wk following seedling emergence.

Tetranycus urticae Bean plants were removed from their growth chamber as needed (about 4 or 5 plants twice each wk). These were placed within cages (31.7 x 30.5 x 5.59 cm; WxDxH) within a second Conviron controlled environment chamber (25°C:20°C; 16L:8D). The top and three sides of each cage were covered in fine screen to permit air movement and prevent escape of mites. Cages were essential for limiting movement of prey mites among plants within the chamber and thereby ensured the establishment of a successional supply of spider mites for rearing *P. persimilis*. Spider mites were transferred among plants by placing several leaves from spider-mite infested plants on previously uninfested plants. Within 2 to 4 weeks, plants became sufficiently infested with *T. urticae* and were used to feed predator colonies.

Phytoseuilus persimilis *Phytoseiulus persimilis* used in earlier studies were reared at the Alberta Research Council, Vegreville, AB where colonies were maintained within rearing cages (40.6 x 4.06 x 80.6 cm; WxDxH). The top and three sides of each cage were covered in 90 μm screen to permit air movement and prevent escape of mites.

Predators from initial shipments were placed on *Tetranychus urticae*-infested bean plants (*Phaseolus vulgaris*) within separate cages. Each cage contained two infested bean plants and predators were fed twice each week by replacing one of the caged plants with a fresh, spider mite-infested plant. Used pots and soil removed from cages were discarded. To prevent movement between predator colonies, each cage was suspended over a moat of soapy water and isolated on separate benches within separate greenhouse compartments. Predators were maintained under quarantine conditions and environmental conditions were controlled (16L:8D; 25°C:20°C). Relative humidity was not controlled and fluctuated between 70 and 90 percent.

P. persimilis used in later studies were transported to the University of Alberta where colonies were established and maintained within growth chambers under controlled environmental conditions (16L:8D; 25°C:20°C). Relative humidity within the incubator was 70±10%; relative humidity within dishes was not measured.

Appendix 2
Stains and staining procedures for light microscopy

Giemsa stock solution

Giemsa powder	1 g
glycerol	60 mL
methanol	66 mL

Add Giemsa to glycerol. Stir for 30 min. Place in a 60°C oven for 30 min to 2 hr. Allow mixture to cool. Add methanol and mix well. Place in a tightly sealed jar. **Alternative method**: Place Giemsa, glycerol, and a magnetic stir rod in a large Erlenmeyer flask. Place flask on a hot plate on low heat and stir constantly. Remove once Giemsa powder and glycerol are completely mixed. Allow mixture to cool and add methanol.

Giemsa working solution (15% Giemsa buffer; pH~6.89)

Giemsa stock solution (above)	27.8 mL
0.5M KH_2PO_4	1.0 mL
0.5M Na_2HPO_4	1.5 mL
distilled water	185 mL

Giemsa staining procedure

1. Air dry smear preparation.
2. Fix in methanol (10 min).
3. Stain in 15% Giemsa buffer (2 hr).
4. Dehydrate in the following ethanol series:
   - 70% ethanol (2 min)
   - 80% ethanol (2 min)
   - 90% ethanol (2 min)
   - 95% ethanol (2 min)
   - absolute ethanol (2 min)
   - xylene (2 min)
5. Mount in Permount.

Appendix 3
Reagents and procedures for transmission electron microscopy

I. Reagents

Fixative: 1% paraformaldehyde, 1.5% glutaraldehyde in 0.12M cacodylate buffer (pH 7.4)

16% formaldehyde (EM grade)	6.25 mL
50% glutaraldehyde (EM grade)	3.0 mL
sodium cacodylate	2.57 g

Combine and add 80 mL distiller water. Adjust pH to 7.4 with concentrated HCl. Add distilled water to bring volume to 100 mL. Store in tightly sealed bottle at 4°C.

0.48M cacodylate buffer stock (pH 7.4)

sodium cacodylate	25.7 g
distilled water	100 mL

Combine in a 500 mL Erlenmeyer flask and swirl until dissolved. Adjust pH to 7.2 with concentrated HCl. Add distilled water to bring volume to 250 mL. Store in a reagent bottle at 4°C.

0.12M cacodylate buffer (pH 7.2)

Dilute 0.48M cacodylate buffer 1:3 with distilled water.

1% osmium tetroxide (OsO_4) in 0.12M cacodylate buffer

4% osmium tetroxide	10 mL
distilled water	20 mL

Dilute osmium tetroxide in distilled water. Add 10 mL of 0.48M cacodylate buffer and mix thoroughly. Store at 4°C in a dark, tightly sealed bottle.

Graded ethanol series

Dilute absolute ethanol with distilled water to produce the following percentages of alcohol: 50, 70, 90, and 100%.

Low viscosity Spurr resin

vinylcyclohexene dioxide (epoxide)	10 g
nonenyl succinic anhydride (hardener)	26 g
diglycidyl ether of polypropylene glycol (plasticiser)	6 g
dimethylaminoethanol	0.2 g

Weigh reagents in a disposable container. Stir for 1 hr. Minimum cure time: 8 hours.

Note: This medium polymerizes with time and becomes unstable when it absorbs moisture. Store excess resin in freezer. Remove from freezer and warm at room temperature for 1 hour before exposing to atmospheric moisture.

II. Infiltration and embedment of mites for transmission electron microscopy

1. Pick up mites individually with a fine paintbrush and place in fixative.
2. Place container under slight vacuum (at room temperature) for 24 hours.
3. Remove fixative with a pipette and replace with 0.12M cacodylate buffer (pH 7.2). Change buffer three times (total time in buffer is 1 hour).
4. Post-fix samples in 1% osmium tetroxide in 0.12M cacodylate buffer (pH 7.2) for 2 hours.
5. Dehydrate and infiltrate as follows:

distilled water	10 minutes
50% ethanol	30 minutes (3 changes in solution)
70% ethanol	30 minutes (3 changes in solution)
90% ethanol	30 minutes (3 changes in solution)
100% ethanol	60 minutes (3 changes in solution)
ethanol:propylene oxide (1:1)	30 minutes (3 changes in solution)
propylene oxide	60 minutes (3 changes in solution)
propylene oxide: Spurr (1:1)	Overnight (16 h minimum.)
pure Spurr	24 hours (minimum)

6. Place samples in flat moulds (1 per mould). Specimens can be moved within the resin by using a thin, round stick (similar in diameter to a toothpick) with ends trimmed to a tapered point by a razor blade. Using the stick, move each specimen as close to the intended block face as possible and orient as desired. This will greatly reduce the need for extensive block trimming once resin blocks have been cured.
7. Cure blocks in a 60°C oven for 16 hours (minimum).

III. Sectioning and staining semi-thin sections

APTS-treated slides

Soak slides for 2 min in a 2% solution of APTS [3-aminopropyltriethoxysiline] in acetone. Dip slides 5 times in distilled water and dry in a 60° oven.
Note: Make and use solution in fume hood. Wear gloves.

Procedure for sectioning semi-thin sections

1. Use a glass knife with an attached boat, or make several knives and provide each with a boat made from metallic tape. Fill boat with distilled water.
2. Rough trim specimen block using a microtome and glass knife. A razor may be used to rough trim under the dissecting microscope followed by additional trimming with a glass knife. Trim block face to a trapezoid shape, the longer side of the trapezoid at the bottom of the block. Trim as close to the specimen as possible.
3. Cut sections from the block by manually advancing the block 1 or 2 μm at a time until the entire face of the block is being cut by the glass knife.
4. Manually advance the block 1.5 to 2 μm (1 to 1.5 μm preferred) to cut semi-thin sections.
5. Pick up sections from water surface using a modified Pasteur pipette (these can be made by heating the fine end and reshaping it into a smooth, ball-shaped end).

6. Float sections onto a small drop of distilled water on a precleaned, APTS-coated slide.
7. Place slide on a hot plate warmed to ~80°C. Add a few drops of chloroform to a filter paper placed within the base of a glass Petri dish. Cover the warming slide with the inverted glass Petri dish.
8. Allow section(s) to dry and remove Petri dish.

1% toluidine blue in borax buffer

toluidine blue	1 g
sodium tetraborate	1 g
distilled water	100 mL

Combine ingredients in a bottle and shake until dissolved. Filter prior to use.

Procedure for staining semi-thin sections

1. Filter toluidine blue stain.
2. Flood sections with a drop of filtered stain and place glass slide on hot plate at medium temperature (~80°C). Remove from heat when edges of stain begin to dry. Do not allow the stain to fully dry on the sections.
3. Wash stain off slide with distilled water from a wash bottle.
4. Rinse with methanol from a wash bottle to remove water and excess stain.
5. Dry on hot plate.
6. Dip in xylene, apply Permount and coverslip.

IV. Cutting and staining ultra-thin sections

Preparation of Formvar films on TEM grids

0.5% Formvar	1 g
ethylene dichloride	100 mL

Combine and stir until dissolved.

Working Solution:

Dilute solution above with an equal volume of ethylene dichloride. Filter before use. Store in brown bottle. The working solution is stable for several months.

Procedure:

1. Filter 0.5% Formvar/ethylene dichloride solution into a thin film casting device.
2. Fill water trough with water.
3. Clean microscope slides by wiping with Kimwipes and place into thin film caster.
4. Immerse slide in Formvar; drain. Note: the slower the solution drains, the thicker the film left on the glass slide.
5. Remove slide from film caster, prop slide at an angle and allow to dry (2-3 min).
6. Etch slide with a razor blade along the top, bottom, and edges.
7. Skim across the surface of the water in trough to remove dust or foreign material.
8. Gently place slide in the water trough at a 45 degree angle to the water surface. This will allow the plastic film to float on the water surface.

9. Check the integrity of the film. Discard film that is too thick (appears yellow) or has holes.
10. Place grids on film surface (dull side down).
11. Lift film and grids from the trough using a piece of paper. Place the paper on top of the film, gently pushing the film into the water. Remove paper.
12. Allow paper and grids to dry in a dust-free location.

Cutting ultrathin sections

1. Trim excess block with glass knife. Maintain trapezoidal shape to block face.
2. Line up block in holder. Position the block carefully so that the widest edge of the trapezoid (block face) is facing bottom.
3. Mount the diamond knife in holder and clamp tightly. Fill boat on knife with distilled water.
4. Slowly advance the knife until it almost touches the block. Adjust the knife so it is parallel with the block face.
5. Cut sections of the desired thickness. Silver or gold sections (~50-150 nm thickness) give the best result. The first few sections may not contain the entire section of tissue and should be removed from the boat using a brush constructed from a single eyelash mounted on a stick.
6. Cut several sections.
7. Dip a glass rod into chloroform and wave over surface of water to stretch and remove small wrinkles from sections.
8. Use brush to group sections. Using forceps to hold each grid, pick up sections on Formvar-coated grids by gently placing the grid below the sections.
9. Allow grids to dry while being held in forceps.
10. Place grids in a tightly sealed Petri dish lined with filter paper.

Staining ultrathin sections

4% uranyl acetate

uranyl acetate	4 g
distilled water	100 mL

Add uranyl acetate to water and stir until dissolved. Filter before use.

lead citrate

lead citrate	1.33 g
sodium citrate	1.76 g
distilled water	30 mL

Combine and shake thoroughly for 1 minute and then intermittently for 30 minutes (solution will appear murky). Add 8 mL freshly prepared 1N NaOH (2 g per 50 mL water) (solution should become translucent). Add distilled water to 50 mL. Mix by inversion.

0.02N sodium hydroxide

Add 0.2 g NaOH pellets to 250 mL distilled water. Shake until dissolved. Store in wash bottle.

Staining procedure

1. Filter uranyl acetate.
2. Place grids in staining tray.
3. Cover grids with distilled water (~ 30 sec).
4. Pour off water and blot excess water with filter paper.
5. Place staining tray in Petri dish and cover grids with uranyl acetate. Place grids in 60°C oven for 20 minutes.
6. Remove staining tray from oven and wash grids with distilled water. Blot dry with filter paper.
7. Place staining tray in Petri dish and surround with NaOH pellets.
8. Use a pipette to cover grids with lead citrate. Cover staining tray immediately with top of Petri dish. Stain for 6 to 7 min. Important: Do not breathe on grids or staining tray when staining with lead citrate. This will cause the lead citrate to precipitate.
9. Pour off lead citrate.
10. Rinse with 0.02N NaOH.
11. Rinse with distilled water and blot dry with filter paper.
12. Remove grids from staining tray and place on filter paper within a small, tightly sealed Petri dish.

Appendix 4
Construction of observation dishes

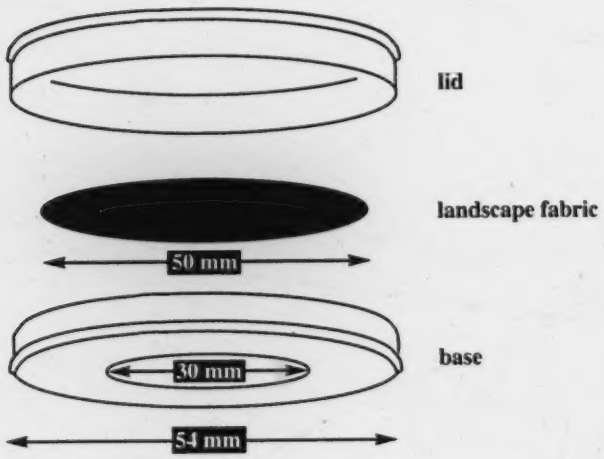


Diagram of an observation dish used in this study.

Observation dishes were made from plastic Millipore dishes, 54 mm in diameter. A 30 mm diameter hole was cut in the base of each dish and covered with 90 μm screen. This permitted air movement and prevented escape of mites. Each dish lid was lined with a moistened, 50 mm diameter disc cut from Filterbond landscape fabric. Excised leaf discs (27 mm in diameter) were cut from bean leaves and placed on moistened fabric discs, the upper leaf surface in contact with the fabric. Observation dishes were inverted, screen side facing downward, to simulate the leaf's natural orientation. Dishes were placed in plastic containers within an incubator under controlled environmental conditions (16L:8D; 25°C:20°C).

Appendix 5
Morphological diagrams of *P. persimilis* life stages (Hesselin, 1976)[†].

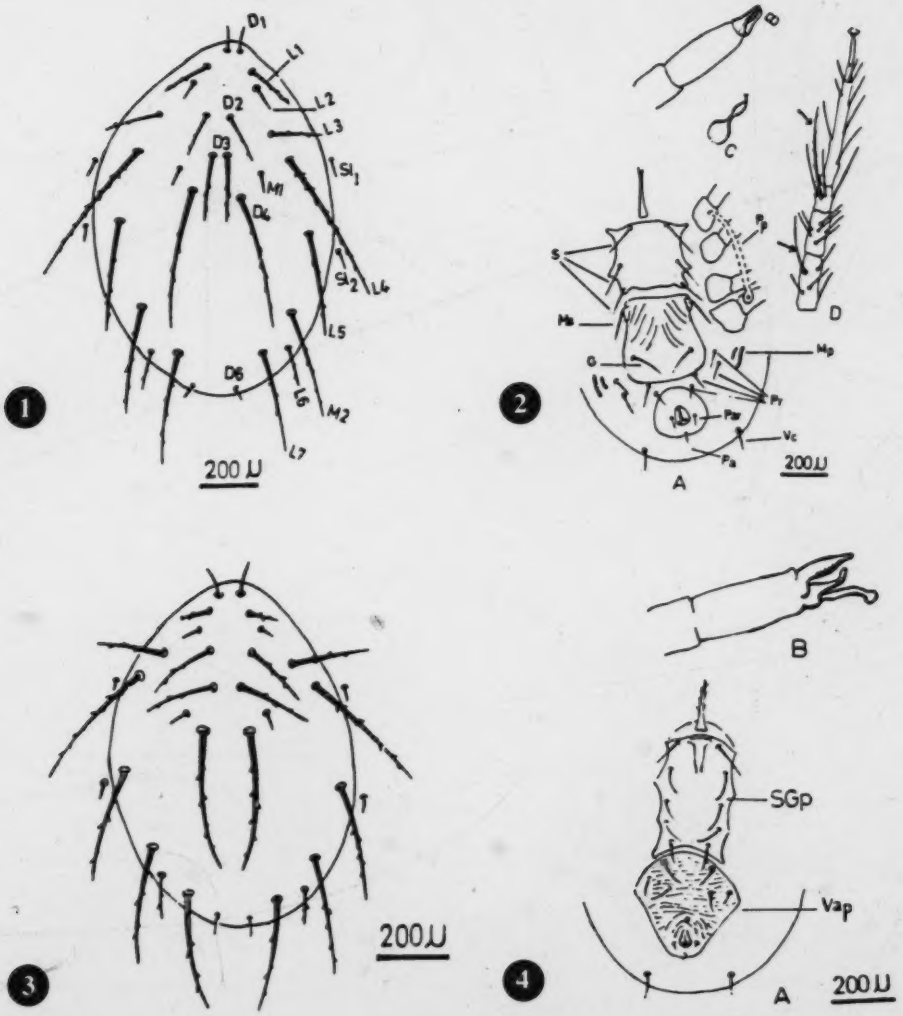
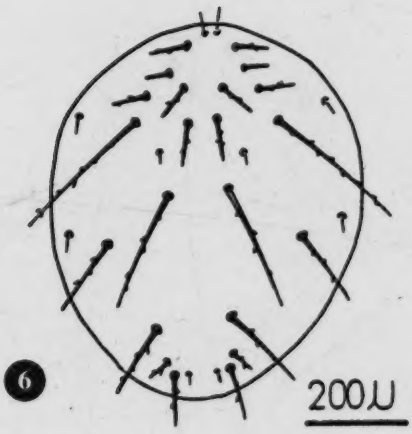


Figure 1. Adult female dorsal shield: D_1-D_4 and D_6, dorsal setae; M_1 and M_2, median setae; L_1-L_7, lateral setae; Sl_1 and Sl_2, sub-lateral setae.

Figure 2. Adult female. A, ventral surface: S, sternal setae; Ms, metasternal setae; G, genital seta; Pr, preanal setae; Par, para-anal seta; Vc, ventrocaudal setae; Mp, metapodal plates; Pa, postanal seta; Pp, peritremal plate. B, Chelicera. C, Spermatheca. D, Arrows indicate macrosetae on fourth leg.

Figure 3. Adult male dorsal shield.

Figure 4. Adult male. A, Ventral surface: sternal and genital plate (SGp), ventri-anal plate (Vap). B, Chelicera.



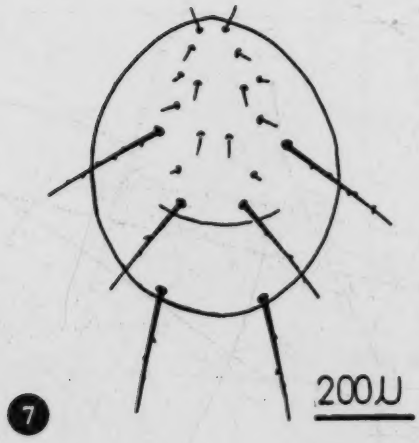


Figure 5. Deuteronymph dorsal shield.
Figure 6. Protonymph dorsal shield.
Figure 7. Larval dorsal shield.

† Hessein, N.A. 1976. Morphological and developmental studies for the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae). *Libyan J. Agric.* **5**, 119-126.